Immune Dysfunction in Egyptian Children with Down Syndrome

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ABSTRACT

Children with Down syndrome (DS) have an increased rate of infection and it has been proposed that this is secondary to derangements of the immune system. Multiple immunologic disturbances were commonly observed in individuals with DS including abnormal proportions of peripheral lymphocytes subsets, cellular dysfunction and others. The present study was conducted on 20 children with Down syndrome. Sex distribution was 9 males to 11 females (1: 1.2) with age ranging from 1 month to 11 years (average 25.9+31.7 months). Control group of 15 healthy children matched for age and sex were included. Karyotype by a standard technique, the evaluation of the immune system by flow cytometry and estimation of serum cytokines (interleukin-1 Beta (IL-1β), interleukin-2 (IL-2) and tumour necrosis factor-alpha (TNF-α)) were carried out. A significant decrease of the absolute number of circulating lymphocytes, a marked significant decrease of B-lymphocytes and dramatic modifications of the T-cell subsets were observed. T-cell subpopulation studies revealed marked decrease in CD4+ subpopulation has been documented with inverted CD4/CD8 ratio, whereas CD8+ cells increased significantly in percentage. A derangement of cells bearing markers associated with natural killing (NK) activity such as CD19+ and CD56+ was observed. Among the most important alterations are the presence of a high number of CD56+ lymphocytes and significantly high percent of CD56+ CD8+ T cells. Regarding cytokine production, it was found that serum IL-2 levels were significantly decreased in DS children compared to the control group and its production was correlated inversely with age. On the other hand; IL-1β and TNF-α levels in DS children showed no significant difference compared to normal subjects. We concluded that thymic alterations and molecular abnormalities due to gene over-expression of loci located on chromosome 21 could be involved. On the other hand, the increased percent of CD56+ CD8+ T cells; as potent antitumour immunity; is emphasizing how rare are solid tumours in DS patients. **(Int. J. Ch. Neuropsychiatry, 2004, 1(1): 65-74)**

INTRODUCTION

Down syndrome (DS) is the most common chromosome anomaly in human beings. Approximately one in 700 live births is born with DS[1]. In addition to the characteristic malformations and mental retardation, impaired immune function is reported in DS patients[2]. The immunodeficiency results in a higher risk of viral infections, with respiratory infections being the most frequent and lower response rates to vaccination. The immunodeficiency appears to start early in the development of the immune system and tends to worsen with ageing of DS individuals[3]. Therefore, DS may be considered as a model of precocious, abnormal ageing of the thymus-dependent system in man.

During the last decade, studies have classified DS as a progeroid, with a process of premature ageing that is responsible for immunological alterations, autoimmune diseases and neoplasms within relatively young age groups compared with the general population[4]. A number of different authors have demonstrated alterations throughout all areas of immunoresponse, irrespective of the clinical expression of these alterations[5,6]. Previous investigations revealed differences in the lymphocyte subpopulations in DS patients when compared with normal subjects[7]. These data suggested an abnormality in the development of the lymphocytes in DS patients.

With the significant progress in caring for DS patients, understanding the underlying causes of immunodeficiency in DS patients remains a challenge. The aim of this study is to evaluate the immunologic disturbances in individuals with Down's syndrome by detecting the immunophenotypic changes in proportions of peripheral blood lymphoid subsets and cytokines levels.

SUBJECTS AND METHODS

This study was carried out on twenty children with Down syndrome (DS). Karyotyping revealed trisomy 21 in all cases. Their ages ranged from 1 month to 11 years with a mean of 25.9+31.7 months. Male to female ratio was 1:1.2 (9 males and 11 females). As a control group, 15 healthy children with a mean age of 30.9+34.6 months (2months to 10 years) were included where male to female ratio was 1.1:1 (8 males and 7 females).

All patients and controls were subjected to the following:

I. Detailed personal, developmental and family history with special emphasis on history of recurrent infection.
II. Thorough clinical examination with special stress on signs of infections at the time of the study.
III. Laboratory investigations:
   1. Complete blood count (CBC) using coulter counter T660 (coulter corporation, Hialeah, Florida, USA) together with examination of leishman-stained peripheral blood (PB) smears for differential leucocytic count.
   2. Immunophenotyping of PB samples by flow cytometry.
   3. Estimation of serum interleukin–1 Beta (IL-1β), interleukin-2 (IL-2) and tumour necrosis factor-alpha (TNF-α) using the enzyme linked immunosorbent assay (ELISA).

- ***Sample collection:***

PB samples were obtained on potassium – ethylene diamine tetraacetic acid (K2–EDTA) with a final concentration of 1.5mg/ml for CBC and immunophenotyping analysis. Serum samples were used to estimate cytokine levels and were stored frozen at –70°c until used.

- ***Phenotypic analysis and flow cytometry:***

Cells labeled with monoclonal antibodies (MoAbs) for cytofluorimetric analysis were prepared from whole blood following standard methods.

MoAbs from Coulter (Immunotech, Coulter Company, Marseille, France) directly conjugated with fluorescein

isothiocyanate (FITC), phycoerythrin (PE), or phycoerythrin cyanin–5 (PC-5) were used to analyze the surface antigens of peripheral blood lymphocytes (PBL).

These MoAbs were: anti–CD3, recognizing all T cells; anti–CD4, reactive with helper/inducer T–cell subset; anti–CD8, recognizing cytotoxic T–cell subset; anti-CD19, reactive with B-lymphocytes; anti–CD56, recognizing the N-CAM molecule, reactive with a subset of cells with natural killer (NK) activity and with a small percentage of CD3+ lymphocytes, which are considered as a subset of cytotoxic T-lymphocytes that mediate non–major histocompatibility complex (MHC)-restricted cytotoxicity. Mouse IgG1 conjugated with FITC, PE or PC5 (Coulter Inc.) was used as a negative control for non specific binding.

These cytofluorimetric analyses were performed using Coulter EPICS XL-Flow cytometer (Coulter, USA). The FACS TAR was equipped with a 15-mw argon laser operating at 486nm wavelength to excite the fluorochromes.

A minimum of 10,000 cells per sample was analyzed. Regarding the value of the percentage of cells stained with each MoAb, for each subject the mean of all determinations performed by the same MoAb conjugated with FITC, PE, or PC5 was calculated.

- ***ELISA for IL-1β, IL-2 and TNF-α:***

Quantitative sandwich ELISA measurement of IL-1β, IL-2 and TNF-α was developed by the modified method described by Park et al.[8]. Serum concentrations of IL-1β, IL-2 and TNF-α were measured with a Human IL-1β ELISA kit, Human IL-2 ELISA kit and Human TNF-α ELISA kit, respectively (Quantikine, R&D systems, Minneapolis, USA). Briefly, each specific monoclonal antibody has been precoated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β, IL-2, or TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for each cytokine was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour develops in proportion to the amount of cytokine bound in the initial step. The colour development is stopped and the intensity of the colour is measured. The plate was read at 550nm using a microplate reader. The concentration of each cytokine was calculated using each recombinant protein as a standard.

Statistical analysis:

This was done according to Ingelfinger et al.[9]. Data were recorded on "investigation report form". These data were transferred to IBM cards using IBM personal computer with the statistical program "SPSS version 8.0 for windows" to obtain mean (x) and standard deviation (+SD). Statistical analyses performed two–tail student's T-test. A two-tailed probability <0.05 was considered statistically significant.

RESULTS

Significant alterations were observed in DS children including the percentage and the absolute number of several PBL subsets (Table 1). DS children presented a significantly decreased number of circulating lymphocytes. T-cell subpopulations studies by flow cytometry revealed marked significant decrease in the absolute number

of circulating CD3+, CD4+ and CD19+ in DS cases compared to the control group. Also, the percentages of CD4+ (helper T-lymphocytes) and CD19+ (B-cells) were significantly low in DS patients. As regards the suppressor-cytotoxic CD8+ lymphocytes and CD56+ cells which are related to NK activity, their percentages were significantly increased in DS cases compared with control group (Table 1 and Fig. 1). Moreover, our results revealed significant increase in percent of CD56+ CD8+ T cells and inverted CD4/CD8 ratio.

Regarding cytokine production, it was found that serum IL-2 levels were significantly decreased in DS children compared to the control group and its production was correlated inversely with age. On the other hand, IL-1β and TNF-α levels in DS children showed no significant difference compared to normal subjects (Table 2 and Fig.2).

Table 1. Comparison of immunophenotypic analysis of peripheral blood lymphocytes (PBL) between DS and control subjects.

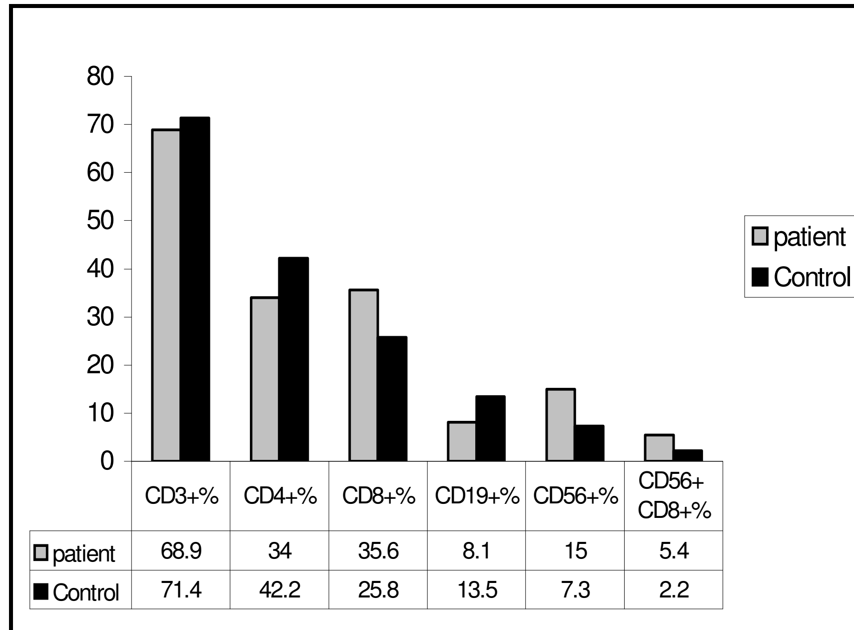
Test		DS children (X+SD)	Control children (X+SD)	P-value
TLC ($X10^9$/L)		8.1+2.8	8.4+2.2	NS
Lymph. ($x10^9$/L)		2.8+1.0	5.0+1.3	<.001
CD3+	**(%)**	68.9+11.1	71.4+8.9	NS
	(n)	191+77	363+128	<.001
CD4+	**(%)**	34.0+10.8	42.2+8.7	.02
	(n)	91+45	216+87	.002
CD8+	**(%)**	35.6+7.5	25.8+5.8	<.001
	(n)	99+44	129+43	NS
CD19+	**(%)**	8.1+4.1	13.5+5.6	.002
	(n)	22+13	130+75	<.001
CD56+	**(%)**	15.0+4.6	7.3+3.4	<.001
	(n)	41+21	35+19	NS
CD56+ CD8+	**(%)**	5.4+3.2	2.2+ 0.9	.001
	(n)	13 + 9	11 + 5	NS

Data are referred to the percentage (%) or to the absolute number $X10^9$/L (n) of PBL positive to MoABs used. NS; non significant

Table 2. Comparison of cytokine levels between DC children and control children.

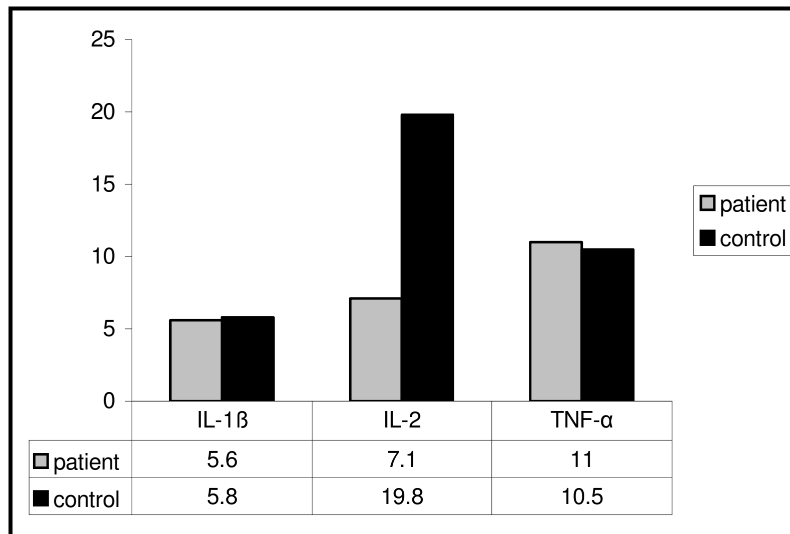
Test	DS children (X + SD)	Control children (X + SD)	P-value
IL-1β (pg/ml)	5.6+1.2	5.8+1.5	NS
IL-2 (pg/ml)	7.1+2.9	19.8+3.7	<.001
TNF-α (pg/ml)	11.0+1.0	10.5+1.1	NS

NS; non significant



	CD3+%	CD4+%	CD8+%	CD19+%	CD56+%	CD56+ CD8+%
patient	68.9	34	35.6	8.1	15	5.4
Control	71.4	42.2	25.8	13.5	7.3	2.2

Fig. (1): Comparison of immunophenotypic analysis of blood lymphocytes between DS and control subjects.



	IL-1ß	IL-2	TNF-α
patient	5.6	7.1	11
control	5.8	19.8	10.5

Fig. (2): Comparison of cytokine levels between DS and control subjects.

DISCUSSION

With the significant progress in caring for DS patients, understanding the underlying causes of immunodeficiency remains a challenge. The present study was carried out to investigate the immunological profile of Egyptian DS children.

In this study, 20 children with Down syndrome were included. Sex distribution was 9 males to 11 females (1: 1.2) with age ranging from 1 month to 11 years (average 25.9+31.7 months) together with 15 healthy children as a control group. Our study revealed that DS children presented a significantly decreased absolute number of circulating lymphocytes, which is consistent with the published studies. In Italy, Bertotto et al.[10] reported that T-lymphocyte proliferation induced by antigen or polyclonal T-cell activators is severely impaired in DS patients. Moreover, lymphocytes in DS patients were shown to have significantly slower synthesis rate of simultaneously active adjacent replicon clusters compared to normal controls. These results demonstrate differences in respect to the structural organization of clusters of replicons between DS and normal lymphocytes. A possible relation of the above phenomenon to the chromosomal radio-sensitivity in DS cells have been reported[11].

There was an evidence of an inefficient release of mature T cells by the DS thymus as Down syndrome thymocytes have a markedly diminished proportion of cells expressing high levels of the alpha, beta T cell receptor (TCR alpha, beta)[12]. Thymus abnormalities were probably caused by superoxide dismutase (SOD-1) hyperexpression in cells, in that reactive oxygen intermediate generation (specifically H_2O_2 production) is enhanced in thymocytes and clearly correlates with apoptosis. Similarly, oxidative injury correlated with the formation of lipid peroxidation by-products and antioxidants which partly inhibit programmed cell death in thymocytes[13]. On the other hand, Park et al.[14] concluded that there is partial impairment of T lymphocytes in aged persons with DS which is significantly higher in males than in females.

The maturation of most T-lymphocyte precursors takes place within the meshwork of thymic epithelial cells. The prothymocytes are positive for the terminal deoxynucleotidyl transferase (TdT) and give rise to cortical thymocytes, which express CD1, CD2, CD3, CD5, and both CD4 and CD8. These CD4 and CD8 double-positive cortical thymocytes differentiate into two lineages: CD4+ or CD8+ lymphocytes of the thymic medulla, by the tenth week of gestation[15].

For cellular immunity assessment, we studied T-lymphocyte subpopulations by flow cytometry. We found out that the absolute numbers of circulating CD3+, CD4+ and CD19+ in DS cases were significantly lower than that of the control group (Table 1 and Fig.1). As regards the percentages of CD4+ and CD19+, they were significantly lower in DS patients. The percentages of suppressor-cytotoxic CD8+ lymphocytes, CD56+ cells; which are related to NK activity; and CD56+ CD8+ T cells; related to antitumour immunity; were significantly increased in DS cases compared to control group with inverted CD4/CD8 ratio.

Previous investigations showed differences in the lymphocyte subpopulations in DS patients when compared with normal subjects[7]. The proportion of CD8 +

lymphocytes was increased in DS patients while that of CD4 + lymphocytes was decreased. The decrease in the CD4 + subpopulation was due to the low proportion of CD4 + CD45RA + naïve lymphocytes in DS patients. It was not clear whether the over-dosage of the CD18 gene plays a role in the abnormal development of lymphocyte subpopulations in the DS patients[16]**.** It was reported that thymic alterations at birth predispose to decrease in peripheral lymphocyte populations, particularly those of lymphocytes CD4+ and CD8+[17]. In Venezuela, significantly diminished helper T lymphocyte (CD4) percentage, helper/cytotoxic (CD4/CD8) ratio and B-cells (CD19) were found in DS patients by matching with control group. An increase in CD8 was also reported by Soto-Quintana et al.[18]. Immune defects were investigated in 43 children with Down's syndrome in Italy. Peripheral T-lymphocytes with the phenotype of helper cells or cluster of differentiation 4 (CD4) were decreased. Circulating activated T cells (CD3/HLA-DR-positive cells) and large granular lymphocytes (CD16/CD56 positive cells) were increased[19]. The functional impairment in DS was not related either to a decrease of circulating mature-type CD3+ cells, which express high levels of surface of CD3 molecules, or to a decrease of the CD4+ subpopulation, but, there is a T cell activation defect, characterized by partial signal transduction through a TCR/CD3 complex, and associated with a selective failure of tyrosine phosphorylation[20]**.**

A recent study in Brazil on cellular immunity assessment in DS patients, lymphocytes CD4+ and CD8+ were counted for 32 patients. CD4+ lymphocyte count was below the fifth percentile for patients' age while, CD8+ lymphocyte count values were above the ninety-fifth percentile in six cases and below the fifth in 3 cases of DS[3]. Cossarizza et al.[4] described reductions in absolute CD4+ lymphocyte numbers increasing with age, with this being the first lymphocyte subpopulation to diminish, in children between 9 and 12 years old.

CD56 is known as neural cell adhesion molecule-1 [21]. It was reported that NK cells, CD56+ T cells in peripheral blood can be a potent antitumour effector after stimulation with interleukin-2 [22]**.** Our results showed significantly increased percentage of NK CD56+ cells in DS cases compared with control group. Moreover, significant increase in percent of CD56+ CD8+ T cells was observed.

As regards cytokine production in DS individuals, our results showed that serum IL-2 levels were significantly decreased in DS children compared to the control group and its production was correlated inversely with age. IL-1β and TNF-α levels in DS children showed no significant difference than normal subjects.

The response to IL-2 has been extensively studied as one potential mechanism underlying the age-related defect in cellular immunity. Several laboratories have demonstrated decreased production of IL-2 after mitogen stimulation, decreased density of IL-2 receptor expression, decreased expression of IL-2 mRNA, and decreased proliferation of T cells in response to IL-2. IL-1 and -2 playing a primary role in activation, recruitment, and proliferation of T lymphocytes. Evidence has been accumulating that there are age-related declines in lymphocyte production and response to other cytokines, such as IL-1 and tumor necrosis factor (TNF)[23].

Cytokine production (IL-1beta, IL-2, IL-6, IL-8, and TNF-alpha), were measured in DS patients in a study done by Park et al.[14]. IL-2 production was significantly decreased in aged individuals with DS. These results are similar to our findings. Interestingly, IL-2 was significantly greater in aged males than in aged females with DS. IL-1beta production was significantly decreased in older adults with DS compared with non-DS controls. Other immune parameters measured in DS were not significantly different from that of age-matched controls.

The status of cytokines in amniotic fluid (AF) from Down syndrome pregnancies was studied. DS amniotic fluid displayed reduced IL-2 bioactivity and lacked IL-2 immunoactivity compared with normal samples and increased tumour growth factor-beta (TGF-β) activity[24]. In another study done by Licastro et al.[19], plasma levels of interleukin-6 were higher in DS children than in controls.

It was reported that, in both DS and control thymuses the mast cells co-localized with TNF-alpha mRNA-expressing cells. In addition, TNF-alpha protein- expressing cells, identified by immunohistochemistry, displayed a granular pattern of staining that is characteristic of mast cells. These results suggested that mast cells may be one source of TNF-alpha in human postnatal thymus. This study demonstrated the constitutive production and location of TNF-alpha in postnatal human thymus. The overexpression of this cytokine in DS thymuses suggested a dysregulation in cytokine production in DS and might provide an explanation for the abnormal thymic anatomy and thymocyte maturation associated with this syndrome[25]. The increased sensitivity of DS thymocytes to TNF may explain anatomical abnormalities in DS thymuses and suggests the involvement of genes encoded on human chromosome 21 in the responses to TNF[26].

Tumor necrosis factor-alpha (TNF-alpha) (6p21.3) is a candidate gene as it interacts with the brain deposition of the amyloid beta-protein (Abeta), one of the neuropathological hallmarks in DS. 136 DS patients and 113 controls were examined by Lucarelli et al.[27] for -850 TNF-alpha polymorphisms. The -850T frequency in DS was significantly higher than in controls. These findings suggest that the -850T allele, which is more common among patients at high risk of dementia such as those with DS, might eventually play a role in the development of dementia.

Alterations of plasma levels of zinc and in the immune system in Down's syndrome (DS) have been reported. These alterations have been associated with a high rate of infectious diseases, which represent the main cause of mortality in affected individuals. Zinc plays a central role in the immune system and has been found to be significantly reduced in people with Down syndrome[28].

In conclusion, the chromosomal error in DS patients involves many genes that encode molecules which are potentially important for the immune responses and lymphocyte development. Interaction between these genes that are affected by the chromosomal anomaly of trisomy 21 might lead to immunological phenotypes that are not expected by simply assuming the gene over-dosage of a single molecule. Our results suggest that more generalized pathological processes, such as early senescence of the immune system or ineffective lymphocyte activation may underlie the immune defects in DS patients.

Our study raises the importance of systematic research into immunodeficiencies in DS patients when the control of morbid associations does not result in a reduction in infectious processes.

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