RESEARCH ARTICLE

Altered serum pro-inflammatory cytokines in children with Down’s syndrome

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ABSTRACT. There are reports showing that pro-inflammatory cytokines are dysregulated in patients with Down’s syndrome (DS). However, most of these reports concern adults. We analyzed cytokine levels in serum samples from children with DS, and compared them with samples from intellectually disabled (ID), and healthy, control children. Blood samples were collected from 24 DS, 24 age-/sex-matched ID, and 24 age-/sex-matched healthy, control children. Serum levels of the cytokines IL-5, IL-10, IL-13, IFN-γ, and TNF-α were measured using a sandwich ELISA method. The age range of the children was 1-15 years, with a mean ± SD of 5.75 ± 4.36 years. TNF-α levels were significantly higher in the DS and ID groups compared with those found in healthy, control children (P < 0.05). The DS and ID groups had significantly higher IFN-γ levels compared with healthy, control children (P = 0.0002 and P < 0.01, respectively), with significant higher levels in the DS than the ID group (P < 0.05). Serum from the ID group showed significantly higher IL-10 levels compared with those from the DS group (P < 0.05), but not the healthy, control group. Significant correlations were found between the differences in TNF-α and IFN-γ levels, in both ID (rs = 0.558; P = 0.005) and DS children (rs = 0.405; P < 0.05). There were no significant differences found in serum levels of IL-13 between the groups, and IL-5 was not detectable in any of the serum samples. Levels of TNF-α and IFN-γ were increased, and IL-10 decreased in serum from children with DS. It may be that these differences contribute to the clinical symptoms seen in DS: consequently, these pro-inflammatory cytokines might be useful as early biomarkers of the disorders associated with DS.

Key words: Down’s syndrome, pro-inflammatory cytokines, TNF-α, IFN-γ

Down’s syndrome (DS) is the most common chromosomal disorder. It is characterized by the presence of an extra copy of chromosome 21 (trisomy 21), which is associated with intellectual disability amongst other things. It is estimated that the incidence of Down’s syndrome is 1 in every 700-1,000 birth world-wide [1]. Different clinical manifestations are associated with DS including heart defects, alterations of the immune system, increased susceptibility to infections, high risk of developing hematological malignancies, phenotypic anomalies, and an Alzheimer-like disease developing many years earlier than seen in the general population.

Changes in certain aspects of the humoral and cellular immune systems in DS have been suggested. Functional impairment of B and T lymphocytes and natural killer cells, and dysfunction of phagocytosis and chemotaxis of polymorphonuclear leukocytes have been shown in different studies [2, 3]. The total number of CD4+ T cells is within the normal range, but CD4+ subsets might be changed and the cytotoxic CD8+ T cell fraction might be increased [4]. B lymphocyte abnormalities also have been demonstrated associated with abnormal levels of IgG and IgM in serum [5]. Ineffective immune responses in DS lead to recurrent viral/bacterial infections and contribute to the development of various pathophysiological symptoms including cognitive impairment [6-8].

Chromosome 21 of human and chromosome 16 of mice carry genes that are involved in the function of the interferon family of cytokines and receptors [9]. Overexpression of chromosome 21-gene products causes changes in levels of inflammatory cytokines in the blood [9]. Two of these inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), have wide-ranging biological effects in the body and important regulatory roles in immune responses. In DS, abnormal pro-inflammatory cytokine production might participate in neuropathological changes such as the Alzheimer’s-like mental retardation associated with DS [6, 10]. It is reported that cells of the
neural system in DS patients are hypersensitive to the anti-
cellular effects of IFNs [11] that are usually associated with
the cognitive impairment seen in DS patients with
increasing the age [12]. While the immune response in DS
seems to be influenced by patient age [13], the major-
ity of studies have evaluated the immune response of adult
patients with DS. In this study, we investigated the possible
early changes in serum proinflammatory cytokine levels in
children with DS and compared them with levels found
in intellectually disabled (ID) and healthy children. Early
changes in proinflammatory cytokine production might be
useful as a biomarker of the clinical disorders seen in DS
and ID patients.

DONORS AND METHODS

Participants and sample collection

The study proposal was reviewed and approved by the
local ethical committee. Potential candidates or their
parents/legal guardians were informed about the study
objectives and procedure, and those who were willing to
participate and sign an informed consent were included.
Twenty four (F/M = 13/11) participants with DS who were
residents of an intellectual disability institute located in
Tehran were included. Karyotypes confirmed trisomy 21
in the children who had been clinically diagnosed with
DS. As the patient control group, 24 age-sex matched
children with ID, other than DS, who were living in the
same institute were included. As the healthy, control
control group, 24 age-sex matched, healthy children who
had been referred to the Children’s Medical Center were
also included. C-reactive protein (CRP) and erythrocyte
sedimentation rate (ESR) were measured for all control
groups.

Serum was separated from blood samples using centrifu-
gation, 800 × g for 10 minutes, which had been collected
for routine laboratory check-ups from each participant.
Portions of the serum samples were stored at -20°C until
use.

ELISA

Serum samples were collected and the levels of interleukin
(IL)-5, IL-10, IL-13, IFN-γ, and TNF-α (Mabtech, Stock-
holm, Sweden) were measured using a sandwich ELISA
method. Briefly, the plates were coated with anti-IFN-
γ/IL-5/IL-10/IL-13 or TNF-α mAb in PBS, pH 7.4, and
incubated at 4 °C overnight. After blocking the wells with
buffer containing PBS plus 0.05% (v/v) Tween 20 and
0.1% (w/v) bovine serum albumin (BSA), serum samples
were added to each well. Biotin-labeled mAb in incuba-
tion buffer was added to each well and as the enzyme,
streptavidin-horseradish peroxidase (HRP) was used.
The reaction was developed using 3,3′,5,5′-tetramethyl
benzidine (TMB) substrate and stopped with 0.5M H2SO4
solution. The plates were washed after each incubation step
using PBS+0.05% (v/v) Tween 20. The plates were read
at 450 nm using a reader (BioTek, Winooski, VT, USA).
The mean optical densities (OD) of wells were compared
with the standard curves prepared using recombinant IL-5,
IL-10, IL-13, IFN-γ, and TNF-α. The cytokine levels
represent the differences between the OD of test and
background wells. The detection limit of the assays was
4 pg/mL for IL-5, 0.5 pg/mL for IL-10, 5 pg/mL for IL-13,
2 pg/ml for IFN-γ and 13 pg/mL for TNF-α.

Statistical analysis

Non-parametric tests of Mann-Whitney and Kruskal-
Wallis, and Dunn’s post-test for paired comparisons were
used for statistical analysis of the data using the SPSS ver-
sion 11.5 (SPSS Inc., USA) and GraphPad Prism version
5.01 (GraphPad Software Inc., USA) software. For corre-
lation, the nonparametric Spearman test was used, and rs
was reported as the correlation coefficient. Nonparametric
tests were chosen because the samples did not follow a
Gaussian distribution. P value of <0.05 was regarded as
significant.

RESULTS

Demographic and clinical characteristics

of participants

The mean age ± SD was 5.75 ± 4.36 years (range: 1-15
years) for each of the three groups.
All participants in the control group had a negative CRP
test and an ESR within the normal range.
The etiology of disabilities and the medical diagnoses of
children with ID were as follows: developmental brain
abnormalities were recorded in most cases of ID. Approx-
imately 3/4 of the brain abnormalities were due to cerebral
palsy (n = 18; 75%), while three cases (12.5%) were
related to cephalic disorders including two cases (8.3%) of
hydrocephaly and one (4.2%) of schizencephaly. Genetic
disorders were diagnosed in two cases (8.3%), and in one
case the etiology of the intellectual disability was unknown
(4.2%).

Cytokine production assay

Using an ELISA method, IL-5, IL-10, IL-13, IFN-γ, and
TNF-α cytokines were measured in serum samples. The
TNF-α levels were significantly higher in serum from the
DS and ID groups compared with those from the healthy,
control group (P<0.05) (figure 1A). Results of IFN-γ mea-
surements showed that serum from the DS and ID groups
had significantly higher IFN-γ levels compared with those
found in the healthy control group (P = 0.0002 and P<0.01,
respectively). However, there was a significant difference
between the DS and ID groups as regards levels of IFN-γ
(P<0.05); significantly higher IFN-γ levels were seen in
the DS group. Serum from the DS group showed signifi-
cantly lower IL-10 levels compared with the ID, but not
the healthy, control groups (P<0.05) (figure 1B). Levels of
IL-13 in serum samples from the three groups were not sig-
nificantly different. IL-5 was not detectable. The changes
in TNF-α levels showed a strong correlation with IFN-γ
(rs = 0.558; P = 0.005), and a strong, inverse correlation
with IL-10 (rs = 0.662; P<0.001) levels in the ID group.
The changes in TNF-α levels showed a moderate corre-
lation with IFN-γ levels (rs = 0.405; P<0.05) in the DS
group. Changes in IFN-γ levels showed a strong, inverse
correlation with IL-10 levels (rs = -0.574; P = 0.003) in
the DS group.
were significantly higher in the DS group compared with those found in the healthy control children (\(P<0.05\)). Levels of IFN-\(\gamma\) were also significantly higher in the DS group than in the ID (\(P<0.05\)) and healthy, control (\(P = 0.0002\)) groups. The changes seen in serum TNF-\(\alpha\) levels correlated significantly with those of IFN-\(\gamma\), in both the ID and DS groups. Higher levels of TNF-\(\alpha\) and IFN-\(\gamma\) cytokines in unstimulated cell-culture supernatant from adult DS patients compared with an ID group were shown in a recent experiment [10]. The overexpression of TNF-\(\alpha\) and IFN-\(\gamma\) mRNA in the thymus of DS patients has been demonstrated previously using in situ hybridization [14]. In agreement with these findings, by intracellular cytokine staining of T cells, a significantly higher number of IFN-\(\gamma\)-producing CD4\(^+\) and CD8\(^+\) T cells have been detected in peripheral blood samples from DS patients than in those from ID and healthy controls [12]. One study in older individuals (>30 years old) with DS showed different results for pro-inflammatory cytokine production [15]. IL-2 production by phytohemagglutinin (PHA)-stimulated monocytes was significantly decreased in older individuals with DS; levels of IL-1b, IL-2, IL-6, IL-8, and TNF-\(\alpha\) were no different from those of age-matched controls [15].

The role of TNF in the regulation of immune cells is known, and the contribution of altered TNF production in various human disorders, including Alzheimer’s disease, cancer and depression has been reported [16-18]. It has been shown that cells in DS patients are hypersensitive to the anti-inflammatory effects of IFNs [11]. The degenerative effects of IFN-\(\gamma\) on neural systems, along with \(\beta\)-amyloid production, has been shown in DS and the trisomy 16 mouse models [19]. These effects usually lead to cognitive impairment in DS patients with increasing the age [12]. In vivo administration of anti-IFN-\(\gamma\) antibody to pregnant mice showed improving effects on the development of trisomy 16 fetuses, and in vitro use of anti-IFN-\(\gamma\) antibody on cell cultures of cortical neurons of trisomy 16 had inhibitory effects on the premature death of these cells [20].

In the current study, significantly lower levels of IL-10 were found in DS children compared to the ID group (\(P<0.05\)). Levels of IL-10 were not significantly increased, but TNF-\(\alpha\) levels were increased in children with DS. Since it is known that IL-10 has an anti-inflammatory effect that inhibits the production of proinflammatory cytokines (such as IL-6 and TNF-\(\alpha\)), so the reciprocal changes in the level of serum IL-10 and TNF-\(\alpha\) are understandable in DS children. In contrast to this study, one study reported increased levels of IL-10 and decreased levels of TNF-\(\alpha\) in children with DS [4].

Abnormal inflammatory cytokines levels were reported in DS patients with hematologic malignancy [21]. Levels of IL-1b, TNF-\(\alpha\), and IFN-\(\gamma\) were found to be different between DS neonates with or without transient abnormal myelopoiesis (TAM). The study revealed that altered levels of cytokine production might have a role in the development of the liver fibrosis and myelofibrosis seen in hematologic malignancy patients with DS [21].

In DS, the consequences of a defective immune response usually manifest later in life, even though the onset of Alzheimer-like dementia in adults with DS might present many years earlier when compared to the general population. Inflammation is involved in the neurodegeneration
[18], and it is suggested that pro-inflammatory cytokines play a role in Alzheimer’s disease, and involve degenerative process of the central nervous system (CNS) in DS [6]. Study of the immune response in children with DS who were not suffering from dementia, i.e. before clinical manifestations of cognitive deterioration, has revealed that significantly higher levels of plasma IL-6, soluble IL-6 receptor (sIL-6R), soluble intercellular adhesion molecule-3 (sICAM-3), soluble vascular cell adhesion molecule-1 (sVCAM-1) and CRP were produced than in control children. Similarly increased levels of IL-6 and CRP were reported in aged patients with Alzheimer’s disease [9]. A correlation between the degree of mental retardation and IL-6 levels was shown in DS patients [6].

In this study, we analyzed serum cytokine levels in DS children with a mean age of 5.75 ± 4.36 years and compared them with age-/sex- matched ID and healthy, control groups. Current data show a high level of TNF-α and IFN-γ in children with DS, and suggest a possible involvement of inflammatory cytokines in early CNS alterations. As regards the correlation between cytokines and dementia, it seems that evaluation of the immune response profile in children with DS is helpful in the early diagnosis of the clinical symptoms seen in adult patients with DS. Further study of the frequency and function of the T lymphocyte subsets in children and adult with DS is recommended.

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REFERENCES


