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Lymphocyte subgroups and recurrent infections in children with Down syndrome – a prospective case control study

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Abstract

Down syndrome (DS) is the commonest genetic disorder and more liable for recurrent infections. We aimed to determine the differences in lymphocyte subgroups between DS children and the healthy population and to study the pattern and likelihood for recurrent infections and hospital admission due to infection. Our study was carried out in the Genetic Unit of Mansoura University Children's Hospital, Egypt. The study enrolled 150 DS (DS group) and 100 controls (CG group). They were assessed for recurrent infections (including tonsillitis, otitis media [OM], pneumonia, upper respiratory tract infections [URTI], sinusitis, and gastroenteritis [GE]) and hospital admission due to infections. All patients were subjected to complete blood count and flow cytometric analysis for expression markers of B lymphocytes (CD19), natural killer (NK) cells (CD56), and T lymphocytes (CD3, CD4 and CD8). We found a statistically significant increase in the frequency of URTIs and sinusitis, OM, pneumonia, and hospital admission in the DS group. As regards the type of recurrent infection in DS, it was highest for URTIs and sinusitis. For age groups below 13 years, a statistically significant decrease in all studied CD markers was found in the DS group, while for the 13-18-year-olds, a statistically significant decrease was found in CD4, CD19, and CD56 in the DS group. Non-significant correlations were found between CD markers and recurrent infection and hospital admission. We concluded that lymphocyte subgroups that carry CD3, CD4, CD8, CD19, and CD56 were decreased in DS. Recurrent infections and hospital admission are still striking feature for DS but are not significantly correlated with lymphocyte subgroups.

Key words: *Down syndrome, lymphocyte subgroups, recurrent infections, CD markers.*

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Introduction

Down syndrome (DS) is one of the commonest genetic conditions worldwide [1] characterised by facial dysmorphic features, congenital heart defects, and hypotonia. In addition, it is associated with increased frequency of haematological malignancies, autoimmune disorders, and recurrent infections [2]. Most of these infections are recurrent respiratory tract infection (RTI) and otitis media (OM) [3], suggesting dysfunction of the humoral immune response. These recurrent infections were found to be one of the most significant health problems in school-aged DS children [4].

Although many differences between the immune system of the general population and that of DS children have been reported, the clinical relevance of these differences is unclear. Various anatomical and medical co-morbidities commonly associated with DS increase infection susceptibility and could also influence immune responses [5]. Several theories about the pathogenesis of immune deficiency in DS have been postulated including over-expression of chromosome 21 genes (e.g. SOD1 and ITGB2) [6], zinc deficiency [7], and increased apoptosis with increased telomeres loss [8, 9].

As regards hospital admission, DS children on average spend two to three times more time in hospital than other children, with more frequent mechanical ventilation and higher mortality rate [10, 11].

Recent studies demonstrated T lymphocytopaenia in all age groups concerning $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ helper cells with the absence of the tremendous expansion that is found normally in early infancy, suggesting defective response to antigenic stimulation. Total T lymphocyte numbers gradually approach the values of the general population with advancing age, but it is doubtful whether these cells have normal function and phenotype [12].

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Severe B cell lymphocytopaenia had been detected in DS patients, with the absence of the normal great expansion in infancy [12]. Moreover, a significant decrease of B cells ($CD19^+$) had been observed in DS foetuses [13]. Another study on subpopulations of lymphocytes in DS showed lower values of CD16, CD3, and/or 56^+ natural killer (NK) cells in all age groups [12].

We conducted a prospective case-control study to determine whether there were differences for lymphocyte subgroups between DS children and the healthy population. This was done by analysis of expression markers of B lymphocytes (CD19), NK cell (CD56), and T lymphocytes (CD3, CD4, and CD8). The second target for our study was to unravel the correlation between these markers and the likelihood of recurrent infections and hospital admission.

Material and methods

Setting

This study was carried out in the Genetic Unit of the Children's Hospital of Mansoura University, Mansoura, Egypt from April 2015 to February 2017. It is one of the most important genetic units in Egypt.

Sample

The study enrolled 150 cytogenetically documented DS patients (DS group) and 100 controls (CG group). The sample was analysed according to age as follows: group I (6-23 months), group II (2-6 years), group III (7-12 years), and group IV (13-18 years). The DS group included 88 males and 62 females, while the CG group involved 55 males and 45 females. DS patients were selected randomly from the inpatient Genetic unit and outpatient Genetic clinics of Children's Hospital of Mansoura University. Inclusion criteria were karyotype-proven DS, including both genders and age groups, from 6 months to 18 years old. Patients with proven congenital heart diseases (echocardiography was done for all cases), those less than six months old, and those maintained on immunosuppressive therapy were excluded from the study. The study was accepted by the Medical Research Ethical Committee of the Medical Faculty of Mansoura University (Code No: MS/832). Written, informed consent was obtained from all the participants' parents or guardians who agreed to be enrolled in the research.

Methods

History

All studied groups were subjected to a thorough history evaluation from medical records and interviews carried out by the physicians, including age, gender, place of residence, maternal age, consanguinity, similar condition, history of recurrent infections (including recurrent tonsillitis, OM, pneumonia, upper respiratory tract infections [URTI], sinusitis and gastroenteritis [GE]) and history of hospital admission due to infections. Patients were labelled to have recurrent tonsillitis if five or more episodes per year were documented [14]. As regards OM, it was considered recurrent if three episodes within six months or four or more episodes during a year [15]. Recurrent URTIs were assumed at 12 or more episodes per year. Patients were considered to have recurrent pneumonia if they developed three or more attacks in one year [16]. Recurrent sinusitis was defined as the occurrence of more than three episodes in one year or the occurrence of chronic sinusitis [17]. Recurrent GE was defined as seven recurrent episodes per a year lasting less than 14 days [18].

Examination

All patients were subjected to clinical examination focusing on general features of DS, anthropometric measurements, cardiac examination, presence of organomegaly, and lymph node enlargement.

Laboratory assessment

All patients were subjected to complete blood count, differential leukocyte count, and chromosomal culture and flow cytometric analysis for expression markers of B lymphocytes (CD19), NK cells (CD56), and T lymphocytes (CD3, CD4, and CD8). CD3, CD4, and CD8 were used as CD markers for total T lymphocytes, helper T lymphocytes, and cytotoxic T lymphocytes, respectively. Flow cytometry analysis was carried out on an FACS Aria III flow cytometer (BD Biosciences, San Jose, USA). The whole lysis method of blood was used [19], and appropriate combinations of monoclonal antibodies (Becton Dickinson Quanti BRITE products) were used to stain peripheral mononuclear cells. The used monoclonal antibodies were clone A3-B1 for CD19, clone MEM-188 for CD56, clone SK7 for CD3, clone B-Z31 for CD8, and clone SK3 for CD4 [20]. Histograms were used to illustrate the results using the FACS Diva software package.

Data analysis

Statistical Package for Social Sciences (SPSS) version 15 was used to analyse data. Qualitative data were presented as percentages and numbers. DS and CG groups were compared using χ^2 test. Quantitative data was presented as mean ±SD. Student t-test was used in comparison of both groups. Correlations between variables were tested using Pearson's correlation coefficient; p-values ≤ 0.05 were considered to be statistically significant.

Results

Descriptive data of sample

The mean ages for the studied CG and DS groups were 6.11 and 5.56 years, respectively. Recurrence of DS in the same family was higher in the DS group than in the CG

Table 1. Descriptive data of the sample

Descriptive data	Control group (n = 100)	Down syndrome group (n = 150)	Significance
Age (years) Mean ±SD	6.11 ±4.34	5.56 ±3.82	t = 1.021 p = 0.309
Gender	n (%)	n (%)	
Male	55 (55)	88 (58.7)	χ^2 = 0.329
Female	45 (45)	62 (41.3)	p = 0.566
Residence			
Urban	54 (54)	70 (46.7)	χ^2 = 1.291
Rural	46 (46)	80 (53.3)	p = 0.256
Similar condition in family	2 (2)	13 (8.7)	χ^{2*} = 4.728 p = 0.03*
Consanguineous parents	17 (17)	18 (12)	χ^2 = 1.246 p = 0.264
Maternal age (years) Mean ±SD	26.01 ±4.12	31.27 ±6.65	t = 7.7150 p < 0.001*

*t – independent t-test; χ^2 – Chi-square test; *p-value significant if ≤ 0.05; χ^{2*}– corrected Chi-square test (Fisher exact test)*

Table 2. Groups differences as regards history of recurrent infections and hospital admission

	Control group n = 100 n (%)	Down syndrome group n = 150 n (%)	Significance
Recurrent tonsillitis	28 (28)	60 (40)	χ^2 = 3.788 p = 0.052
Recurrent URTIs and sinusitis	36 (36)	76 (50.7)	χ^2 = 5.219 p = 0.022*
Recurrent OM	4 (4)	35 (23.3)	χ^{2*} = 17.033 p < 0.001*
Recurrent pneumonia	3 (3)	25 (16.7)	χ^{2*} = 11.268 p = 0.001*
Recurrent GE	29 (29)	47 (31.3)	χ^2 = 0.154 p = 0.694
Hospital admission	5 (5)	27 (18)	χ^2 = 9.085 p = 0.003*

*URTIs – upper respiratory tract infections; OM – otitis media; GE – gastroenteritis; χ^2 – Chi-square test; *p-value significant ≤ 0.05; χ^{2*}– corrected Chi-square test (Fisher exact test)*

Table 3. Groups differences as regards complete blood count and differential leucocyte count

Complete blood count parameters	Control group (n = 100)	Down syndrome group (n = 150)	Significance
Platelet (PLT/mm^3) Mean ±SD	302250 ±93894.78	269986.67 ±80860.97	t = 2.811 p = 0.005*
Haemoglobin (gm/dl) Mean ±SD	10.95 ±1.37	10.68 ±1.38	t = 1.533 p = 0.127
WBCs (cell/mm^3) Mean ±SD	9000.53 ±1262.36	5553.2 ±791.71	t = 24.307 p < 0.001*
Neutrophils (cell/mm^3) Mean ±SD	4178.2 ±847.05	3099.67 ±621.79	t = 10.922 p < 0.001*
Lymphocytes (cell/mm^3) Mean ±SD	4135.6 ±890.27	1834.8 ±347.1	t = 24.627 p < 0.001*
Monocytes (cell/mm^3) Mean ±SD	436.8 ±60.13	368.07 ±85.1	t = 7.48 p < 0.001*

*t – independent t-test; *p-value significant ≤ 0.05*

group (8.7% vs. 2%, p = 0.03). Also, maternal age was significantly increased in the DS group (mean maternal age was 31.27 years for the DS group and 26.01 years for the CG group, p < 0.001). A non-statistically significant difference between both groups was found as regards age (p = 0.309), gender (p = 0.566), residence (p = 0.256), and consanguinity (p = 0.264) (Table 1).

Group differences as regards history of recurrent infections and hospital admission

Significant increases in the frequency of URTIs and sinusitis (p = 0.022), OM (p < 0.001), and pneumonia (p = 0.001) were found in the DS group. Non-statistically significant differences were shown between the CG and DS groups as regards frequency of tonsillitis (p = 0.052) and GE (p = 0.694). As regards hospital admission, it was significantly higher in the DS group than in the CG group (p = 0.003). As regards the type of recurrent infection in the DS group, it was highest for URTIs and sinusitis (50.7%) followed by tonsillitis (40%), GE (31.3%), OM (23.3%), and lastly pneumonia (16.7%) (Table 2).

Groups differences as regards complete blood count and differential leucocyte count

Statistically significant decreases in WBC count (p < 0.001), neutrophil count (p < 0.001), total lymphocyte count (p < 0.001), monocyte count (p < 0.001), and platelet count (p = 0.005) were detected in the DS group. No statistically significant difference was shown between the DS group and the CG group regarding haemoglobin (p = 0.127) (Table 3).

Groups differences as regards CD markers of B and T lymphocytes and natural killer cells in different age groups

As regards groups I, II, and III, a statistically significant decrease in all studied CD markers was found in the DS group when compared with the CG group (p < 0.001).

Table 4. Differences in CDs markers of B and T lymphocytes and natural killer cells between different age groups of Down syndrome and control groups

CD markers	Group I		Group II		Group III		Group IV	
	DS (n = 25)	CG (n = 22)	DS (n = 75)	CG (n = 35)	DS (n = 38)	CG (n = 32)	DS (n = 12)	CG (n = 11)
CD3	1450.8 ±324.49	2545 ±346.38	1161.47 ±374.25	2624.86 ±524.3	1107.37 ±295.85	1949.38 ±398.19	1315.83 ±311.96	1610.91 ±370.42
t-value	11.177		14.842		9.883		2.073	
p-value	< 0.001*		< 0.001*		< 0.001*		0.051	
CD4	668 ±139.46	1447.73 ±191.78	500.93 ±164.84	1521.43 ±353.82	460 ±159	1097.81 ±245.45	574.17 ±168.28	892.73 ±225.79
t-value	16.074		16.26		12.636		3.859	
p-value	< 0.001*		< 0.001*		< 0.001*		< 0.001*	
CD8	782.8 ±226.23	1120 ±241.31	657.87 ±236	1100.57 ±203.82	650 ±183.76	845.31 ±168.18	750 ±182.16	718.18 ±158.54
t-value	4.942		9.554		4.604		0.445	
p-value	< 0.001*		< 0.001*		< 0.001*		0.661	
CD19	416.4 ±126.09	1348.18 ±345.96	412.13 ±116.15	1256 ±134.58	423.95 ±116.98	783.13 ±79.25	165.83 ±114.13	540 ±53.1
t-value	11.953		33.72		15.228		9.919	
p-value	< 0.001*		< 0.001*		< 0.001*		< 0.001*	
CD56	185.6 ±76.33	954.55 ±316.95	230.67 ±91.18	476 ±43.33	231.84 ±96.89	475.31 ±95.71	259.17 ±63.6	984.55 ±151.42
t-value	11.1		19.128		10.531		15.22	
p-value	< 0.001*		< 0.001*		< 0.001*		< 0.001*	

*t – independent t test; *p-value significant ≤ 0.05*

While for group IV, statistically significant decreases were found in CD4, CD19, and CD56 ($p < 0.001$) in the DS group when compared to the CG group. As regards CD3 and CD8, statistically non-significant changes were found ($p = 0.051$ and 0.661 respectively) (Table 4).

Correlations between CD markers and hospital admission and sum of recurrent infections in the Down syndrome group

Non-significant negative correlations were found between CD19 and CD56 and recurrent infections ($r = –0.05$, $p = 0.545$; and $r = –0.07$, $p = 0.396$, respectively). Also, insignificant negative correlations were found between CD19 and CD56 and hospital admission ($r = –0.06$, $p = 0.565$; and $r = –0.08$, $p = 0.41$, respectively). Non-significant positive correlations were found between CD3, CD4, and CD8 and recurrent infections ($r = 0.05$, $p = 0.55$; $r = 0.022$, $p = 0.79$; and $r = 0.062$, $p = 0.458$, respectively). Insignificant positive correlations were found between CD3, CD4, and CD8 and hospital admission ($r = 0.051$, $p = 0.555$; $r = 0.026$, $p = 0.8$; and $r = 0.07$, $p = 0.51$, respectively) (Table 5).

Table 5. Correlations between CDs markers and recurrent infections and hospital admission in Down syndrome group

	Recurrent infections		Hospital admission	
CDs markers	r	p	r	p
CD19	–0.05	0.545	–0.06	0.565
CD56	–0.07	0.396	–0.08	0.41
CD3	0.05	0.55	0.051	0.555
CD4	0.022	0.79	0.026	0.8
CD8	0.062	0.458	0.07	0.51

r – Spearman correlation coefficient; p significant if ≤ 0.05

Discussion

Down syndrome children are more liable for have recurrent infections, autoimmune disorders, and leukaemia, suggesting immune dysfunction [21]. We conducted a prospective case-control study in a cohort of Egyptian DS children, aiming at evaluating the CD profile of these patients regarding B lymphocytes (CD19), NK cells (CD56), and T lymphocytes (CD3, CD4, and CD8). We also tried

to unravel the correlations between these markers and the likelihood of recurrent infections and hospital admission due to infections.

Our study revealed that the DS group had a significant increase in recurrent infections in the form of URTIs and sinusitis, OM, and pneumonia, with a significant increase in hospital admission over the CG group. However, both groups showed no statistically significant difference regarding recurrent tonsillitis and GE. Our findings are supported by de Vries [22], who documented that immunodeficiency in DS children is commonly presented by recurrent URTIs and chest infections in infancy and early childhood. Our findings also go hand in hand with a study from the Netherlands done on 22 DS children and their healthy siblings, which found a higher frequency of RTIs ($p = 0.001$) with more hospitalisations ($p = 0.007$) among the DS group. An insignificant difference between both DS and healthy siblings was observed in this study regarding frequency of GE ($p = 1$) [23].

As regards the pattern of infection in our study, it was higher for URTIs, followed by tonsillitis, GE, OM, and lastly pneumonia. This pattern is similar to a recent study in which the frequency of URTIs was the highest (84%) [21]. This finding supports the idea that recurrent URTIs are still a significant health problem among DS children and require more attention. URTIs increase hospital admission and are associated with a lower health-related life quality [3, 4, 24].

In our study, there was significant decrease in total leucocyte count, neutrophil count, and total lymphocytes and monocytes. This is in agreement with Bloemers *et al.* [25], who found in their study of 82 cases (41 control and 41 DS) that there were significantly lower counts of total lymphocytes, total leukocytes, granulocytes, and monocytes in the DS group than in the general population. Also, prior literature reports are in agreement with our study [12, 26, 27]. From our study, we found that the core of immune dysfunction in DS could be related to alteration of these parameters. However, Zampieri *et al.* [28] reported a significant decrease in total lymphocyte count but no significant difference in the count of neutrophils and monocytes when compared with controls.

CD3 count was significantly decreased in the DS group in age groups under 13 years, while there was a non-significant difference in CD3 levels in age groups over 13 years, supporting the theory that with increasing age the difference in the count of total T lymphocytes (CD3) between the DS and general population decreases, and thus apoptosis is not the cause of immune-dysfunction in DS. This finding is supported by Faddan *et al.* [29], who assessed apoptosis markers using flow cytometry in peripheral lymphocytes in 30 cases (15 DS and 15 control) and found an insignificant tendency towards apoptosis in DS.

As regards CD4, a statistically significant decrease was described in all age groups. This is in agreement with a study from the Netherlands in which $CD4^+$ invariant NK T cells, $CD4^+$ T lymphocytes, and $CD4^+CD25^{high}$ T cells were significantly decreased in DS children compared to their siblings [23]. Moreover, a more recent study reported that levels of CD4, CD19, and CD3 were significantly lower in a DS group (85 patients and 64 controls, $p < 0.05$) [30]. On the other hand, Cetiner *et al.* [31] found higher values of CD4 in DS patients.

Another important finding in our research was the significant decrease in CD8 in age groups under 13 years old in DS. This finding is in contrast to the hypothesis of a link of recurrent infection in DS with continuing anti-inflammatory state in DS. Cetiner *et al.* [31] reported in their study a higher percentage of CD8 in 32 DS children and linked the recurrent infection in their DS group to higher CD8 values.

CD19 count in our study was significantly lower in DS patients in all ages. Our finding is similar to Seckin *et al.* [21], who studied B lymphocyte dysfunction in 39 DS patients and 37 healthy children and found a significant decrease in CD19 in DS (1061 ±195 vs. 1594 ±410, $p = 0.001$). A reduced number of B lymphocytes has also been shown in DS in several studies [30, 32, 33]. These studies were carried out in DS children above the age of six years. In common with the findings of the previous studies, the results of our study strongly suggest that DS children have an intrinsic defect of B cell differentiation, causing a significant decrease in the numbers of effector B cells.

Furthermore, a significant decrease in CD56 count was found in the DS group ($p < 0.001$) in all studied age groups. Our finding is in agreement with several authors who described a significant decrease of cells with high and intermediate NK activity phenotype ($CD16^+$ cells and $CD56^+$), while cells having low NK activity phenotype ($CD31^+$ and $CD30^+$) were significantly increased in DS [34]. On the other hand, an increased percentage of CD56 in DS children was documented in many reports [26, 31, 35].

In the DS group, correlations between B lymphocyte subsets and recurrent infections and hospital admission due to infections were insignificant negative correlations. This goes hand in hand with the study by Verstegen *et al.* [33], who assessed lymphocyte subpopulations in relation to recurrent RTIs and autoimmune diseases in 95 DS children and 33 control children and did not find a relation between B lymphocyte subpopulations (CD19) and the incidence of allergy or infections in DS. A more recent study found insignificant correlation between CD19 complex expression and infection frequency [21].

In our study, there were insignificant positive correlations between expression markers of T lymphocytes and recurrent infections and hospital admission due to infection. This is in agreement with Kusters *et al.* [6] who found

no association of low count of T cells and the occurrence of recurrent infections.

There were insignificant negative correlations between NK cells and recurrent infections and hospital admission. This is supported by Ribeiro *et al.* [16], who evaluated risk factors associated with recurrent infections in DS and found limited value of NK cell dysfunction as a risk factor.

From our study and other previous literature data, we support that recurrent infections and hospital admission in DS might be associated with other factors than immune dysfunction, including co-morbidities such as abnormal anatomy of airways and ears, macroglossia, inability to handle secretions, and reactive airway disease.

Conclusions

In conclusion, the immune status of DS is completely different from that of healthy children. Reduced numbers of lymphocyte subgroups that carry CD3, CD4, CD8, CD19, and CD56 were evident in DS. Recurrent infections and hospital admission are still striking features for DS but are not significantly correlated with lymphocyte subgroups. Our study added data concerning expression markers of immune cells in DS that could be valuable to literature. Several extended studies using more expression markers are needed to improve our knowledge about the immune profile of DS and to understand the exact factors related to immune dysfunction in DS.

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The authors declare no conflict of interest.

References

1. Morris JK, Springett A (2014): The National Down Syndrome Cytogenetic Register for England and Wales: 2010 Annual Report. London Public Heal Engl.
2. Kusters MAA, Verstegen RHJ, Gemen EFA, De Vries E (2009): Intrinsic defect of the immune system in children with Down syndrome: a review. Clin Exp Immunol 156: 189-193.
3. McDowell KM, Craven DI (2011): Pulmonary complications of Down syndrome during childhood. J Pediatr 158: 319-325.
4. Cruz N V, Mahmoud SA, Chen H, et al. (2009): Follow-up study of immune defects in patients with dysmorphic disorders. Ann Allergy Asthma Immunol 102: 426-431.
5. Ram G, Chinen J (2011): Infections and immunodeficiency in Down syndrome. Clin Exp Immunol 164: 9-16.
6. Kusters MAA, Gemen EFA, Verstegen RHJ, et al. (2010): Both normal memory counts and decreased naive cells favor intrinsic defect over early senescence of Down syndrome T lymphocytes. Pediatr Res 67: 557-562.
7. Lott IT (1982): Down's syndrome, aging, and Alzheimer's disease: a clinical review. Ann N Y Acad Sci 396: 15-27.
8. Cuadrado E, Barrena MJ (1996): Immune dysfunction in Down's syndrome: primary immune deficiency or early senescence of the immune system? Clin Immunol Immunopathol 78: 209-214.
9. Holmes DK, Bates N, Murray M, et al. (2006): Hematopoietic progenitor cell deficiency in fetuses and children affected by Down's syndrome. Exp Hematol 34: 1611-1615.
10. Megged O, Schlesinger Y (2010): Down syndrome and respiratory syncytial virus infection. Pediatr Infect Dis J 29: 672-673.
11. Watts R, Vyas H (2013): An overview of respiratory problems in children with Down's syndrome. Arch Dis Child 98: 812-817.
12. De Hingh YC, Van Der Vossen PW, Gemen EFA, et al. (2005): Intrinsic abnormalities of lymphocyte counts in children with down syndrome. J Pediatr 147: 744-747.
13. Zizka Z, Calda P, Fait T, et al. (2006): Prenatally diagnosable differences in the cellular immunity of fetuses with Down's and Edwards' syndrome. Fetal Diagn Ther 21: 510-514.
14. Ghosh S, Feingold E, Dey SK (2009): Etiology of Down syndrome: Evidence for consistent association among altered meiotic recombination, nondisjunction, and maternal age across populations. Am J Med Genet Part A 149: 1415-1420.
15. Morris JK, Mutton DE, Alberman E (2005): Recurrences of free trisomy 21: analysis of data from the National Down Syndrome Cytogenetic Register. Prenat Diagn 25: 1120-1128.
16. Ribeiro L, Jacob C, Pastorino AC, et al.(2003): Evaluation factors associated in recurrent and/or severe infections in patients with Down's syndrome. J Pediatr (Rio J) 79: 141-148.
17. AlKhater SA (2009): Approach to the child with recurrent infections. J Fam Community Med 16: 77.
18. Perry S, De la Luz Sanchez M, Hurst PK, Parsonnet J (2005): Household transmission of gastroenteritis. Emerg Infect Dis 11: 1093.
19. Bossuyt X, Marti GE, Fleisher TA (1997): Comparative analysis of whole blood lysis methods for flow cytometry. Cytometry 30: 124-133.
20. Jalla S, Sazawal S, Deb S, et al. (2004): Enumeration of lymphocyte subsets using flow cytometry: Effect of storage before and after staining in a developing country setting. Indian J Clin Biochem 19: 95-99.
21. Seckin AN, Ozdemir H, Ceylan A, Artac H (2017): Age-related alterations of the CD19 complex and memory B cells in children with Down syndrome. Clin Exp Med 18: 125-131.
22. De Vries E (2006): Patient centred-screening for primary immunodeficiency: a multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol 145: 204-214.
23. Broers CJM, Gemke RJBJ, Weijerman ME, et al. (2012): Frequency of lower respiratory tract infections in relation to adaptive immunity in children with Down syndrome compared to their healthy siblings. Acta Paediatr 101: 862-867.
24. Verstegen RHJ, Gameren-Oosterom HBM, Fekkes M, et al. (2013): Significant impact of recurrent respiratory tract infections in children with Down syndrome. Child Care Health Dev 39: 801-809.
25. Bloemers BLP, van Bleek GM, Kimpen JLL, Bont L (2010): Distinct abnormalities in the innate immune system of children with Down syndrome. J Pediatr 156: 804-809.
26. Cossarizza A, Monti D, Montagnani G, et al. (1990): Precocious aging of the immune system in Down syndrome: Alteration of b lymphocytes, T-lymphocyte subsets, and cells

with natural killer markers. Am J Med Genet Part A 37(S7): 213-218.
27. Cocchi G, Mastrocola M, Capelli M, et al. (2007): Immunological patterns in young children with Down syndrome: is there a temporal trend? Acta Paediatr 96: 1479-1482.
28. Zampieri BL, Biselli-Périco JM, de Souza JES, et al. (2014): Altered expression of immune-related genes in children with Down syndrome. PLoS One 9: e107218.
29. Faddan NA, Sayed D, Ghaleb F (2011): T lymphocytes apoptosis and mitochondrial membrane potential in Down's syndrome. Fetal Pediatr Pathol 30: 45-52.
30. Yılmaz C, Dogan M, Başarslan F, et al. (2015): Evaluation of Lymphocyte Subgroups in Children With Down Syndrome. Clin Appl Thromb 21: 546-549.
31. Cetiner S, Demirhan O, Inal TC, et al. (2010): Analysis of peripheral blood T-cell subsets, natural killer cells and serum levels of cytokines in children with Down syndrome. Int J Immunogenet 37: 233-237.
32. Verstegen RHJ, Driessen GJ, Bartol SJW, et al. (2014): Defective B-cell memory in patients with Down syndrome. J Allergy Clin Immunol 134: 1346-1353.
33. Verstegen RHJ, Kusters MAA, Gemen EFA, De Vries E (2010): Down syndrome B-lymphocyte subpopulations, intrinsic defect or decreased T-lymphocyte help. Pediatr Res 67: 563-569.
34. Ugazio AG, Maccario R, Notarangelo LD, Burgio GR (1990): Immunology of Down syndrome: a review. Am J Med Genet Part A 37(S7): 204-212.
35. Tolmie JL (1996): Down syndrome and other autosomal trisomies. In: Rimoin DL, Connor JM, Pyeritz RE, Korf BR (eds.). Emery and Rimoin's Principles and Practice of Medical Genetics (3rd ed.). Churchill Livingstone, New York; 925-971.