Pneumonia and Impaired T Cell Function in Children with Down's Syndrome: Double Strike

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Abstract: Down's syndrome (DS) is the most common chromosomal abnormality in humans and is the most common known genetic cause of intellectual disability. DS is known for increased incidence of respiratory infections and autoimmune diseases, indicating impaired immunity. Subjects and methods: This study included sixty seven children; 49 preschool children with DS, with ages ranging from 2 to 6.5 years and 18 healthy, age- and gender-matched controls. Free T4, TSH, and thyroid autoantibodies (anti-thyroglobulin and anti-TSH receptor antibodies) were measured. Evaluation of total leucocytic count (TLC), lymphocytes, CD3+, CD4+, CD8+ and CD56+ cells was performed for each subject. Sputum specimens were collected from all DS subjects and controls for microscopic examination and culture. Results: Among 49 DS child 23 (46.9%) had signs and symptoms of respiratory tract infection, 11 of DS children (22.4%) were suffering from pneumonia. The culture results of sputum samples revealed that staphylococcus aurous was the most common organism; it represents 37.9% of the total bacterial pathogen isolates and 45.4% of the pneumonic patient's isolates. Nineteen DS subjects (38.78%) were hypothyroid according to the thyroid profile tests. Thyroid autoantibodies were detected in five (10.2%) of DS children, one euthyroid and four hypothyroid children. The values of TLC, lymphocyte, CD3+ and CD4+ cells $(5772.2 \pm 1861.1/mm^3, 2234.2 \pm 597.8, 1774.2 \pm 396.5$ and 760.9 ± 298.4 respectively), were lower in DS children than healthy controls $(7908.0 \pm 1464.8/mm^3, 3158.9 \pm 722.5, 2252.0 \pm 636.8$ and 1389.3 ± 379.4 respectively) and the differences were statistically significant. CD8+ and CD56+ cells were higher in DS children $(979.4 \pm 285.2$ and 393.2 ± 102.9 respectively) than healthy controls $(741.8 \pm 170.6$ and 175.5 ± 52.8 respectively) with significant statistical differences. CD4/CD8 ratio was reversed in DS children (0.78 ± 0.27). In conclusion, respiratory tract infection is very common in DS children and can easily complicate to pneumonia because of the complex impairment of T-lymphocytes which is one of the reasons of the defective immune responses among DS children. Staphylococcus aureus was the most common organism causing pneumonia in children with DS. Annual screening for thyroid function and thyroid autoantibodies in preschool DS children is very important to prevent further intellectual deterioration and improve overall development.

1. Introduction

Down syndrome (DS) is the most common chromosomal abnormality among live-born infants and is the most frequent cause of mental retardation in man [3,16]. Respiratory tract infections are the most important cause of mortality in individuals with DS at all ages [4]. Acute respiratory tract infections especially pneumonia is a common reason for hospitalization and morbidity in children with DS [4,10,13].

In DS, non-immunological factors including structural abnormalities of the airways and lungs, glue ears, obstructive sleep apnoea and gastro-oesophageal reflux, may play a role in the increased frequency of respiratory tract infections [20]. Oropharyngeal aspiration (OPA) of food and fluids is known to be associated with pneumonia in dysphagic children with neurological disease and direct causality is often assumed [17,29]. Secondary immunodeficiency due to metabolic or nutritional factors in DS, particularly zinc deficiency, has been postulated [7,20].

Autoimmune phenomena such as acquired hypothyroidism, coeliac disease and diabetes mellitus occur at higher frequency compared with non-DS subjects. Leukemia is estimated to be 15–20 times more frequent in DS [11,16,24]. The most common autoimmune disease in DS is related to the thyroid gland. Thyroid autoantibodies were found in 13–34% of subjects with DS [14,15,23,28]. It is remarkable that the prevalence of thyroid autoantibody increases with age, being common after the age of 8 years [14,15]. However, **_Shalitin and Phillip (2002)_** described two infants in whom chronic autoimmune thyroiditis was diagnosed at the age of 5 and 8 months with DS [23].

The increased susceptibility to infection, malignancies, and autoimmune diseases, suggests that immunodeficiency is an integral part of DS [1,10].

In recent decades several studies have been performed to elucidate abnormalities of the immune system in DS [4]. The abnormalities of the immune system associated with DS include: mild to moderate T and B cell lymphopenia, with marked decrease of naive lymphocytes, impaired mitogen-induced T cell proliferation, reduced specific antibody responses to immunizations and defects of neutrophil chemotaxis[16,20]. The molecular mechanisms leading to the immune defects observed in DS individuals and the contribution of these immunological abnormalities to the increased risk of infections require further investigation [20].

In the current study we aimed to investigate some indicators of T cell immune response, such as white blood cells, total lymphocytes and some indicators of T helper and supressor cells, to study thyroid function and the presence of thyroid autoantibodies; and above all the life threating pneumonia infection in preschool DS children.

2. Subject and Methods

This study was conducted on sixty seven pediatric children divided into two groups: Group 1 comprised 49 children with Down's syndrome, 32 males and 17 females their ages ranged from 2 to 6.5 years (3.5 ± 1.6). DS children were present at the time of the researchers' visit to Early Detection & Intervention Unit of Ahmed Maher Teaching hospital and pediatric out-patient clinics of Ain Shams universty Hospitals. They were particepated in the study after getting written informed consent from parents of each child. The research was conducted between January 2009 and Desemper 2010.

The diagnosis of DS children depends on clinical features and was confirmed by chromosomal analysis of Trisomy 21. Group II comprised 18 heathy children; 10 males and 8 females their ages ranged from 3 to 7.3 years (3.8 ± 1.7). Control subjects were from out-patient clinic coming for triviel symptoms, they were in good general conditions, and had no acute or chronic diseases affecting the immune system. The two groups were matching in age and sex, and they were all subjected to the following:

Complete medical history and clinical examination were done for DS children and control subjects with a special consideration for symptoms and signs of pneumonia like fever, chills, rigor, myalgia, malaise, diarrhoea, cough and dyspnoea. In addition to lake of air space (consolidation) on chest X-ray [4]. And symptoms and signs of hypothyroidism, mainly constipation, dry skin, prolonged sleep and increased body weight; as identified by using Down's syndrome growth chart

[18] and WHO child growth standards, heigth and weight for age [30].

Specimen collection:
I-Blood specimens:

A convenience sample, not less than five ml of venous blood, was collected aseptically from each child. Blood samples were delivered to the laboratory in three aliquots. One ml of blood was anti-coagulated with disodium EDTA for processing in automated hematology cell coulter for blood count. Two ml were anti-coagulated with tripotasium EDTA to be analyzed by flowcytometry for the levels of CD3+, CD4+, CD8+ and CD56+ cells in a FACS Count Flow-cytometer (Becton & Dickinson, USA). And two ml of clotted blood for assay of TSH, free T4 and thyroid auto-antibodies (anti-thyroglobulin and anti-TSH receptor antibodies).

Total leucocytic count (TLC) and lymphocytic counts: were carried on Sysmex hematology coulter, Sysmex Corporation, Cobe, Japan.

Flowcytometric analysis of peripheral blood leucocytes: Monoclonal bodies (MoAbs) 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).The following monoclonal antibodies were used; anti-CD3 (anti-Leu4) recognizing all T cells, anti-CD8 (anti-Leu2a) recognizing cytotoxic T-cell subset, anti-CD4 (anti-Leu3) reactive with helper inducer T-cell subset, anti-CD56 (anti-Leul9) recognizing the N-CAM molecule reactive with resting and activated CD16+ cells, and with a small percentage of CD3+ lymphocytes, which are considered a subset of cytotoxic T lymphocytes that mediate non-major histocompatibility complex (MHC) restricted cytotoxicity. Four cytometry tubes were used for each subject in the study. About 100 μL of EDTA treated blood was added to each of the previous tubes. (Becton Dickinson, San Jose, CA, USA).

TSH and free T4 assays were carried by chemilumenescence Kit (Roch Diagnostics, US).The chemiluminescence's immunoassay test utilizes a unique monoclonal antibody directed against a specific antigenic determinant on the hormone molecule. Hypothyroidism was diagnosed on the basis of a combination of a raised serum concentration of thyroid stimulating hormone (TSH) (reference values range from 0.2-4.2 IU/L) and or a marginally low concentration of free T4 (reference values range from 12-22 pmol/ ml) combined with

the presence of symptoms and signs associated with hypothyroidism.

Thyroid auto-antibodies detection: anti-thyroglobulin and anti-TSH receptor auto-antibodies were detected using a sensitive solid-phase immunosorbent radioassay kit (Biomedical diagnostic division, Solon, Ohio, US) based on binding the human ^{125}I-labeled antigens to the autoantibody. If antibodies were present in a titer of ≥5, the sample was considered positive.

II-Sputum specimens:

Spontaneously expectorated sputum specimens were collected from the subjects in a sterile screw capped containers and transported as soon as possible within 2 hours of collection for culture. Sputum samples were processed by adding an equal volume of sputolysin (sputosol , Unipoth , Hampshire , UK)and incubated for 30 minutes at 37ºC during which they were vortexed for 5-10 seconds.

Microbiological examination of sputum specimens:(Cheesbrough 2000)[6]
(I) Microscopical examination :
- Wet preparation of the sputum examined microscopically by the low power to asses appropriateness of the sample .Only good quality sample were cultured .If large numbers of squamous epithelial cells were detected, the specimen was unsuitable for culturing as it was mostly saliva .
- Gram smear was done for pus cells and bacteria.
- Potassium hydroxide (KoH) preparation when fungal infections were suspected.
- Giemsa smears when pneumonic plaque or histoplasmosis was suspected
- Eosin preparation: to examine sputum for eosinophils.
- Ziehl-Neelsen smears to exclude acid fast bacilli.

(II) Culture on different media:
- Blood agar with optochin disc, incubated at 37 aerobically.
- Chocolate agar incubated in 5-10% CO2 for H. influenzae.
- MacConkey agar for Gram-negative bacilli.
- Lowenstein Jensen media (Oxoid) was used to exclude *Mycobacterium tuberculosis (M.TB)* .The decontaminated specimens with N-acetyle-L-cystein-sodium hydroxide (NALC-NaOH) were inoculated on the medium and incubated for 6-8 weeks before being discarded as negative. Medias were incubated in a slant position with the screw cap loose for at least a week until the sediment has been adsorbed *(Springer et al 1996).[25]*

Statistical analysis:
Statistical analysis of the data was performed with *SPSS*, version 11.0 software, and continuous variables were presented as means and SD. Comparison of qualitative variables of DS group and the control group were carried out using the fisher's exact test. Student's t-test was used to compare quantitative variables. P value of ≤ 0.05 was considered significant.

3. Results
The present study was conducted on 67 children divided into 2 groups; Group 1 comprised 49 children with Down's syndrome, 32 males and 17 females their ages ranged from 2 to 6.5 years (3.5 ± 1.6). Group II comprised 18 healthy children; 10 males and 8 females their ages ranged from three to 7.3 years (3.8 ± 1.7). The two groups are matching in age and sex. Their weight and height data is represented in table 1. Weight of DS chidren was on 90th centile and 75th centile for males and females respectively on plotting on Down's chart in comparison with controls who lied on 50th centile for males and females. As regards to height, DS children were on 25th centile and 50th centile for males and females respectively while that of controls were on 50th centile for both males and females. (Table 1)

Among the 49 DS children, 23 (46.9%) had signs and symptoms of acute respiratory tract infection, 11 of the DS children suffering from pneumonia (22.4%). All of of the pneumonic patients were admitted to the pediatric department of the university hospital. Regarding the culture results of sputum samples, 29 bacterial pathogens had been isolated from sputum samples of the 23 DS patients but none of the controls. Staphylococcus aureus was the most common organism; it represents 37.9% (11/29) of the total culture isolates and 45.4% (5/11) of the pneumonic patient's isolates. Klebsiella pneumonia 24.1% (7/29) and 8.1 % (2/11), Candida albicans represents 17.2% (5/29) and 18.1% (2/11), Haemophilus influenza 10.3% (3/29) and 9.1% (1/11). While Streptococcus pyogenes, Streptococcus pneumoniae and Pseudomonas aeruginosa represent 3.4% (1/29) of the total isolates. The pathogenic organisms were isolated from sputum of 20 of the DS patients with acute respiratory tract infection; 10 of the pneumonic patients were included.

Atypical pneumonia was diagnosed in one child; he was suffering from severe signs and symptoms of pneumonia with abdominal pain, diarrhea and confusion, his sputum culture showed no growth on the used media and he did not respond to empirical antibiotic treatment. He was admitted to the

intensive care unit (ICU) in the university hospital but he passed away. (Table 2)

TLC /mm^3 was lower in DS children (5772.2 ± 1861.1 /mm^3) than in normal controls (7908.0 ± 1464.8/mm^3) with significant statistical difference (P<0.005). Lymphocyte count/mm^3 was lower in DS children (2234.2 ± 597.8 /mm^3) than in healthy controls (3158.9 ± 722.5 /mm^3) with significant statistical difference (P< 0.005). CD3+ lymphocytes absolute values were lower in DS children (1774.2 ± 396.5) than in healthy controls (2252.0 ± 636.8/mm^3) with a significant statistical difference (P<0.05).

Absolute values and percents of CD4+ lymphocytes were found lower in DS children (760.9 ± 298.4 and 33.9 % ± 12.5) than in healthy controls (1389.1 ± 379.4 and 45.8% ± 4.6) and statistical difference were significant (P<0.005 for both absolute count and percents). CD8+ lymphocytes absolute values and percents were higher in DS children (979.4 ± 285.2 and 45.6% ± 15.6) than in healthy controls (741.8 ± 170.6 and 23.5% ± 2.6) with statistical significant difference (P<0.05 and 0.0001 respectively). CD4/CD8 ratio was lower in DS children (0.78 ± 0.27) than healthy controls (1.8 ± 0.19) with significant statistical difference (P<0.0001). CD56+ cells count and percents were higher in DS subjects (393.2 ± 102.9 and 18.8% ± 6.8) than healthy controls (175.5 ± 52.8 and 5.2% ± 1.7) with significant statistical difference (P<0.0001) for both absolute count and percents). (Table 3, Figure 1)

On studying thyroid function, 19/49 (38.78%) of children with DS were hypothyroid, but none of the control group revealed any abnormality in thyroid function. Anti-thyroglobulin auto-antibodies were detected in five (10.2%) DS children; one euthyroid and four hypothyroid and in none of the control group and anti-TSH receptor auto-antibodies were not detected in any of DS children or the normal control group. (Table 4)

Table (1): Weight and height data of Down's syndrome children

Group	Down's syndrome children.		control children	
Gender	Males (no.32)	Female (no.17)	Males (no.10)	Female (no.8)
Weight	13.9± 4.6 (90^{th*})	10.9± 1 (75^{th*})	14.7± 1.7(50^{th*})	13±1.4 (50^{th*})
Hight	83.2± 8 (25^{th*})	80.1±5 (50^{th*})	91.7± 10.1(50^{th*})	88.3± 6.1(50^{th*})

*centile

Table (2): Culture results of DS respiratory tract infection and pneumonia infection.

Group	Respiratory tract infection (no. and %)	Pneumonia infection (no. and %)
Staphylococcus aureus	11(37.9%)	5 (45.4%)
Klebsiella pneumonia	7 (24.1%)	2 (18.1 %)
Candida albicans	5 (17.2%)	2 (18.1%)
Haemophilus influenza	3 (10.3%)	(9.1%)
Streptococcus pyogenes	1 (3.4%)	0 (0%)
Streptococcus pneumoniae	1 (3.4%)	0 (0%)
Pseudomonas aeruginosa	1 (3.4%)	0 (0%)

Table (3): Immunological profile of the studied subjects

Group	Down's syndrome No.49, (mm^3)	Healthy controls No.18, (mm^3)	t	P
TLC	5772.2 ± 1861.1	7908.0 ± 1464.8	3.150	<0.005**
Lymphocytes	2234.2 ± 597.8	3158.9 ± 722.5	3.563	<0.005**
CD3+ count	1774.2 ± 396.5	2252.0 ± 636.8	2.353	<0.05*
CD4+count	760.9 ± 298.4	1389.3 ± 379.4	4.716	<0.005**
CD4 %	33.9 ±12.5	45.8 ± 4.6	2.969	<0.005**
CD8+ count	979.4 ± 285.2	741.8 ± 170.6	2.442	<0.05*
CD8 %	45.6 ± 15.6	23.5 ± 2.6	4.590	<0.0001**
CD4/ CD8	0.78 ± 0.27	1.8 ± 0.19	11.860	<0.0001**
CD 56 count	393.2 ± 102.9	175.5 ± 52.8	6.320	<0.0001**
CD56%	18.8 ± 6.8	5.2 ± 1.7	6.252	<0.0001**

TLC: Total leucocytic count.   *=Significant.   **= Highly Significant .   † Insignificant (P>0.05).

Table (4): Thyroid function in Down's syndrome children and healthy controls

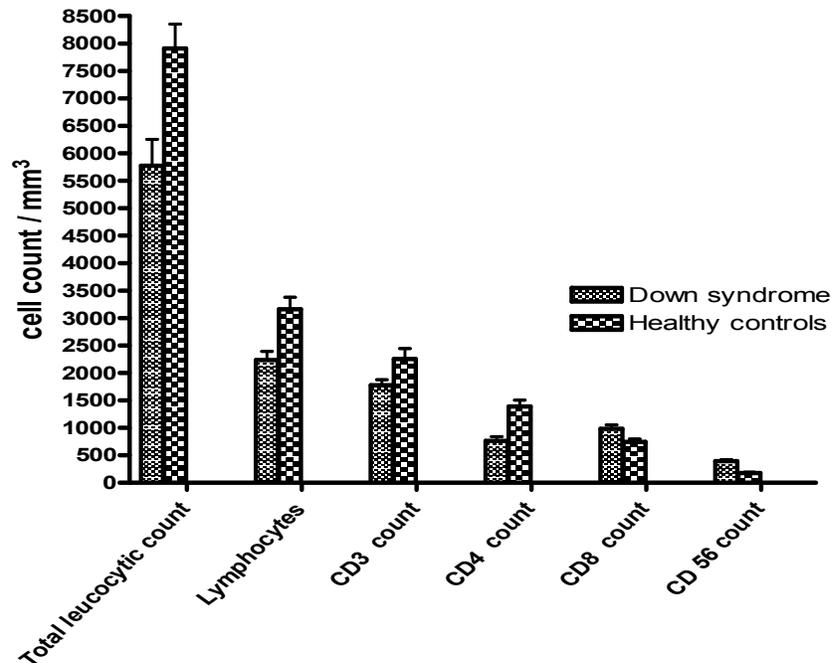| Group	DS children (no.49)	Controls (no.18)
Hypothyroid	19 (38.78%)	0 (0%)
Anti-thyroglobulin	5 (10.2%)	0 (0%)
Anti-TSH receptor	0 (0%)	0 (0%)

Figure (1): Total leucocytic count, lymphocytes, CD3+, CD4+, CD8+ and CD56+ cells in Down's syndrome children and healthy controls.

4. Discussion:

Down syndrome (DS) is the most common genetic disease and is presented with cognitive impairment, cardiac and gastrointestinal abnormalities, in addition to other miscellaneous clinical conditions [20]. DS individuals may have a high frequency of infections, usually of the upper respiratory tract, characterized by increased severity and prolonged course of disease, which are partially attributed to defects of the immune system [20,22].

The present study revealed the high incidence of respiratory tract infection among DS preschool children, 23/49 (46.9%), 11/49 (22.4%) of them were suffering from pneumonia. One child, with sever pneumonic condition, was admitted to the ICU, then passed away. It is widely accepted that children with multisystem involvement demonstrated a higher association with pneumonia than nornals [29]. In agreement with our results, **Hilton et al. (1999) [13]** comprehensively reviewed 232 hospital admissions among DS children over 6 year period, and found that lower respiratory tract pathology was the most common cause for acute hospital admission. Based on age groups, the highest percentage of admissions their studies were among 1–5-year-old children (45%). The predominant diagnosis of admission to the ICU was pneumonia (18%). It was found that Staphylococcus aureus was the most common organism; it represents 37.9% of our total culture isolates and 45.4% of the pneumonic patient's specimens. However, **Alonso et al.(2005)[2]** had reported bacterial pathogens isolates from tracheal secretion culture in 58 % of their DS studied group and the most common pathogen was Staphylococcus aureus (42 %). Three children (25 %) developed lobar pneumonia.

The increased frequency of haematological malignancies, autoimmune diseases and infections in DS, and the observed high frequency of hepatitis B surface antigen carriers, had already led to the hypothesis that DS is associated with abnormalities of the immune system [16,20]. The basis of the immune defects is still unclear [4]. The current study detected a significant decrease of the TLC in DS children. Some studies noted that in DS children, the immune cellular status is similar to the normal population [7,21]. Peripheral blood mononuclear cells from DS subjects are characterized by several alterations leading to a

decreased response to infection, and a decreased killing ability of microorganisms. This may be one reason for the decreased immunity seen in children with DS [1,21].

In accordance with some earlier studies, we detected a significant decrease of the absolute number of circulating lymphocytes. In agreement with *Cossarizza et al.(1990)* [9], we confirmed the presence of a significant reduction of CD3+ lymphocyte absolute number in DS children. It has been noted that there is an inefficient release of mature T cells by the DS thymus, associated with selective failure of tyrosine phosphorylation [21,26].

We found that DS children had less T helper cells than the control group [8,21]. Some studies documented a normal proportion of CD4+ T cells, whereas the percentage of suppressor-cytotoxic CD8+ lymphocytes is markedly increased and CD4/CD8 ratio of patients with DS and normal controls were similar [5,19]. On the contrary, *Corsi et al. (2003)* [8] showed that peripheral CD4+ T cells were lower in children with DS, whereas mean values of cytotoxic CD8+ T cells were comparable with those from healthy children. *Cocchi et al. (2007)* [7] noted that the immune cellular status in DS children is similar to the normal population as far as white blood cell, lymphocyte, CD4 +, CD8 +, natural killer and immunoglobulins. *Guazzarotti et al. (2009)* [12] documented significant increase of both CD4+ and CD8. In our study, absolute values and percents of CD4+ lymphocytes were lower and CD8+ cells were higher in DS children than control group. This was concomitant with *Corsi et al. (2003)* [8], who reported a decrease in CD4+ and an increase in CD8+ cells with inverted CD4/CD8 ratio.

CD56+ cells are considered the cells capable of non MHC-restricted cytotoxicity, and appear to play an important role as tumor infiltrating lymphocytes [5]. The current study showed that CD56+ cells percents and absolute values are higher in DS children than the control group. It was noted that the expansion of this small subset could be the consequence of an effort of DS immune system to compensate for the functional and numerical reduction of other cytotoxic subsets [5,9].

Many of immunological alterations in DS subjects are similar to those characteristic of chromosomally normal subjects of advanced age, DS subjects are aging fast [3,16]. Reduced thymic endocrine activity [20] and the zinc deficiency characteristic of DS [7] and molecular abnormalities due to gene over expression of loci located on chromosome 21 might be responsible for the derangement of T and NK subsets [10,12]. It was suggested that zinc sulfate supplementation improves thyroid function in hypozincemic DS children [7,20].

Thyroid dysfunction, particularly hypothyroidism, is very common in DS. Intrestingly, *Unachak et al.(2008)* [28] claimed that sub-clinical hypothyroidism is very common in children with DS. *Tüysüz and Beker (2001)* [27] reported prevalence of congenital hypothyroidism as 1.8% in children with DS, 25.3% of them had compensated hypothyroidism.The prevalence of hypothyroidism varies among different investigators from 3–54% in subjects with DS of all ages [14,15,23,28]. In our work, we detected hypothyroidism in 38.8% of DS group and none of the controls. The variation in prevalence between different studies might be related to the age variation among the studied subjects and/or to differences in diagnostic criteria.

In the present work, thyroid autoantibodies were detected in 10.2% of DS children, four hypothyroid and one euthyroid. In contrast to our results, *Karlsson et al. (1998)* [15], reported hypothyroidism in 30 DS subjects, half of them acquired the condition before the age of 8 years, but only one of them displayed thyroid autoantibodies at diagnosis. They claimed that, autoimmune thyroid disease is uncommon in young children with DS but is common after 8 years of age

Conclusion

Respiratory tract infection is very common in DS children and can easily complicate to pneumonia because of the complex impairment of T-lymphocytes subsets, which is one of the reasons of defective immune responses among DS children. Staphylococcus aureus was the most common organism causing pneumonia in children with DS. Annual screening for thyroid function and thyroid autoantibodies in DS preschool children is very important to prevent further intellectual deterioration and improve overall development. The awareness of the breadth of respiratory problems and a plan to monitor subjects with DS for their development have the potential to improve outcomes, as some of these conditions are readily identifiable and able to be treated.

A major re-appraisal in attitudes towards DS is required to ensure that the medical and social needs of people with the disorder are adequately met across their entire lifespan, accompanied by the provision of appropriate levels of care and management.

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