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Immunological Markers in Children with Genetic Disorders and Recurrent Respiratory Tract Infections

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Abstract

BACKGROUND: Recurrent respiratory tract infections (RRI) are one of the extremely high common reasons for pediatric visits and hospitalization. Immunodeficiencies are considered as important conditions that may increase the probability of occurrence of RRI. Mannose-binding lectin (MBL2) is a protein of the innate immune system involved in the opsonization and the complement activation. MBL2 deficiency is associated with infectious diseases mainly chest infections; however, subnormal MBL2 levels are also seen in healthy subjects. Primary immunedeficiencies are associated with recurrent infections which mainly appear in early childhood.

AIM: The aim of the study was to estimate T and B and natural killer cells percentage and to investigate the MBL2 and immunoglobulins (Igs) serum levels in children with recurrent RRIs in different genetic disorders compared to normal control.

METHODS: This study included 50 children having a history of recurrent RRIs. All patients had genetic disorders and referred to National Research Centre for follow-up, in addition to, 25 children, age- and sex-matched as a healthy control group. They were subjected to full clinical examination and laboratory investigations including complete blood count (CBC), CD3, CD4, CD8, CD16, and CD19 by flow cytometry, MBL2 by enzyme-linked immunosorbent assay (ELISA), and Igs serum concentrations by nephelometry.

RESULTS: CD16 showed a non-statistical significant difference between both patient groups. Serum levels of IgA in patient groups showed a significant decrease compared to the control group. Moreover, the serum level of IgM results shows a highly significant decrease when compared with the control group. There was no statistically significant difference in MBL2 and IgG serum levels between patient groups and control group.

CONCLUSION: Children with genetic disorders and recurrent RRIs showed a statistically significant decrease of IgA and IgM serum levels as compared to the control group, while the serum level of MBL2 did not show significant results.

Introduction

Respiratory tract infections (RRIs) are a worldwide problem, and one of the first causes of morbidity and mortality in childhood, the causes of susceptibility are poorly defined. Recurrent RRI is the most common sign of the ten warning signs of primary immunedeficiency (PID) [1]. Recurrent respiratory infections in childhood influence the bronchoalveolar and the vascular development of the lungs. This causes many long-term complications. The early treatment, depending on the etiology, has to be initiated. The World Health Organization data demonstrated that children may present, annually, during their first 5 years of their life, with 4–8 incidents of respiratory infections that affect mainly lower respiratory system. Respiratory infections are considered as recurrent diseases from three incidents of acute infections during a 6 months period [2].

Mannose-binding lectin (MBL) is a serum protein, a pattern recognition molecule of the innate immune defense. It is protein belongs to the collectin family, which includes surfactant proteins A and D, with a parallel function and structure. It binds carbohydrates as mannose which is found on the surfaces of many pathogens that make the activation of the complement pathway so facilitating phagocytosis. Direct opsonization similarly occurs through collectin receptors on the phagocyte surface. Many studies showed that MBL2 variants could be weakly associated with increased susceptibility to numerous infections [3]. MBL deficiency is considered as the main inherited immunodeficiency in humans, with an incidence of 5% homozygote and 30% heterozygote [4]. Deficiency is associated with susceptibility to recurrent infections usually as upper respiratory, abscess, meningococcal disease, and sepsis [5], [6]. Low serum levels of MBL2 are accompanied by perinatal infections [7].

Immunoglobulins (Igs) are essential mediators of humoral immunity by neutralization, opsonization, and phagocytosis of the pathogens as well as the complement activation. Immunodeficiency patients are

typically evaluated by the clinical presentation and early screening tests with (complete blood count [CBC]), Ig levels, and estimation of lymphocyte subsets [8]. Early diagnosis of PIDs can improve their outcome and quality of life, and help to prevent debilitating complications [9]. Immunophenotyping by flow cytometry and quantization of peripheral blood lymphocyte subsets could be either diagnostic or prognostic useful in numerous PIDs but not all [10].

PID is frequently associated with recurrent infections presenting in early childhood and is mainly caused by non-virulent microorganisms [11]. Antibody-mediated immune system deficiencies are considered an important risk of recurrent infections, especially the RRI by encapsulated bacteria [12], [13]. Several studies reported an association between low level of Igs, disease severity, and unfavorable outcomes in patients with sepsis [14].

The aim of this study is to estimate MBL2 and Igs levels in patients with recurrent RRI either with decreased immune cells or normal levels of immune cells as compared to the normal control.

Methods

This study included 50 patients. They had different genetic disorders and were presenting with recurrent chest infections and attending Clinical Genetics Clinic National Research Centre for follow-up. This research was approved by the Medical Ethical Committee of the National Research Centre, Egypt (ethics number; 16108), according to the World Medical Association Declaration of Helsinki. Written consent was taken from each patient guardian. All patients and controls were subjected to full medical history and clinical examination. Laboratory investigations, including CBC and immune cells measurements by flow cytometry revealed altering immune profile for some of them. After flow cytometry estimation of T, B lymphocytes and natural killer (NK) cells, they were categorized into two groups, Group 1; n = 25 patients with a recurrent chest infection and low T, and or B cells and or NK and Group 2; n = 25 recurrent chest infections and normal level of T, B, and NK cells, in addition to 25 children as a healthy control group (Group 3), age- and sex-matched.

Blood samples were obtained from all patients during the first contact with the physician, and at the time of diagnosis, and serum was separated and stored at −80°C until analysis.

Flow cytometry studies

Peripheral blood CD3%, CD4%, CD8%, CD19%, and CD16% for all patients were estimated according to manufacture steps using monoclonal antibodies (BD Biosciences, USA) by flow cytometry procedure [15]. Isotype-matched controls were performed for every analysis for the evaluation of possible non-specific staining and autofluorescence.

Estimation of MBL2 level

Serum levels of MBL2 in patients and controls were measured by ELISA using the AviBion Human MBL2 ELISA kit (Ani Biotech Oy, Vantaa, Finland) as the manufacturer's protocol.

Estimation of serum Igs levels

Serum IgA, IgM, and IgG levels in patients and controls were monitored by nephelometry immunoassay using (Minineph^{TM}, the Binding Site Ltd, PO Box 11712, Birmingham, B14 4ZB, U.K) as the manufacturer's protocol.

Statistical analysis of data

Data are expressed as mean ± SD. Statistical significance of the difference was analyzed using SPSS version 20. $p < 0.05$ was considered statistically significant.

Results

This study included 50 patients, their age ranged from 1 year to 9 years; they were 29 males and 21 females, in addition to, 25 healthy control subjects' age- and sex-matched (Table 1).

Table 1: Age and sex in the three groups

Parameters	Group 1 (n=25)	Group 2 (n=25)	Control (n=25)
Age range (years)	1–6	2–9	1–9
Sex			
Males	14	15	19
Females	11	10	6

There was a highly statistically significant difference between Group 1 and Group 2 as regard CD3 ($p = 0.0029$) and statistically significant difference with CD4, CD8, and CD19 ($p = 0.0052$, 0.0301, and 0.0265), respectively (Table 2).

Table 2: Flow cytometry estimation of T, B, and NK cells Mean±SD

Parameters	Group 1 (n=25)	Group 2 (n=25)	p-value
CD3%	35.39±21.26	54.39±8.81	**0.0029**
CD4%	21.11±9.91	32.88±10.05	**0.0052**
CD8%	10.46±6.21	17.23±8.82	**0.0301**
CD19%	11.70±9.21	17.94±5.31	**0.0265**
CD16%	14.55±6.84	16.17±6.39	0.5589

$p<0.05$. NK: Natural killer, SD: Standard deviation.

Serum levels of IgA in Group 1 and Group 2 showed a significant decrease ($p = 0.0114$ and 0.0316, respectively) from the control group, while IgM results show a highly significant decrease in both groups

(p = 0.0032 and 0.0024, respectively) as compared to the control group but non-statistically significant difference in IgG and MBL2 (Table 3).

Table 3: Serum MBL2 and immunoglobulins levels (Mean±SD) in the three studied groups

Parameters	Group 1 n=25	Group 2 n=25	Group 3 (Control) n=25	p-value (Group 1 and C)	p-value (Group 2 and C)
MBL2 (ng/ml)	3.16±3.78	1.38±0.77	2.89±2.40	0.8840	0.0856
IgA (g/L)	0.42±0.34	0.73±0.29	1.31±0.98	**0.0114**	**0.0316**
IgM (g/L)	0.57±0.36	0.75±0.24	1.41±0.56	**0.0032**	**0.0024**
IgG (g/L)	7.56±2.18	9.01±2.04	9.62±3.20	0.1398	0.5985

p<0.05. MBL2: Mannose-binding lectin 2, Ig: Immunoglobulin, SD: Standard deviation.

Discussion

MBL is a liver-derived complement-activating protein; it acts as an opsonin that recognizes repetitive sugar molecules present in many species of microorganisms. The MBL2 gene has several common polymorphisms that can affect the function or concentration of the protein [16].

The current study included 50 children patients with age range from 1 year to 9 years, referred to Clinical Genetics Clinic, National Research Centre with a history of recurrent chest infections. After flow cytometry estimation of CD3%, CD4%, CD8%, CD19%, and CD16%, the patients in this study were divided into two groups: Group 1, n = 25, 14 males and 11 females, with associated immune cells defects and Group 2, n = 25, 15 males and 10 females without associated immune cells deficiency in different genetic disorders. The study had a control Group 3 of 25 healthy children with age- and sex-matched (Table 1).

Flow cytometry is a highly sensitive method for evaluating the immune system and facilitating the diagnosis of PID [10]. Our results revealed a statistically significant decrease in CD4%, CD8%, and CD19% in Group 1 as compared to Group 2 ($p < 0.05$). CD3 showed a high statistically significant decrease in Group 1 than Group 2 ($p < 0.005$). CD16 showed no statistically significant difference between groups ($p > 0.05$) (Table 2). Stepensky *et al*. found a decrease in CD4% and not CD16% during his study that was done on severe combined immunodeficiency [17]. This is in agreement with Shearer *et al*. stated that the principal immunologic change documented in multicenter, longitudinal studies were that CD4+ T-lymphocytes decreased rapidly in infected infants and children [15].

Our study demonstrated no significant difference between MBL2 serum levels in patients having recurrent chest infection either with or without associated immune cells deficiencies in different genetic disorders patients in both groups Group 1 and Group 2, respectively, compared to the control group (Group 3) ($p > 0.05$) (Table 3). This is in agreement with Atan *et al*. who demonstrated no statistically significant correlation between MBL2 genotype and the occurrence of recurrent RRI in children [18]. Furthermore, our results are in agreement with Jørgensen *et al*. who studied MBL in both recurrent respiratory infection and severe combined immune deficiency groups; they suggested that MBL is not increased in patients with immunological dysregulation than in healthy subjects [19]. In another study population, MBL deficiency had no association with microbial etiology. Low levels of Igs and MBL2 were not associated with the etiology, nor the severity, or the outcome in community-acquired pneumonia [20]. Ishii *et al*. postulated that MBL2 deficiency is not a risk factor for severe life-threatening infections [21].

MBL2 variant alleles are associated with an increased risk of infections [22]. Koch *et al*. stated that MBL2 plays an important role in acute respiratory infection in vulnerable children aged 6–17 months [23]. Another population-based study did not find any significant differences in infectious disease in MBL2-deficient adult patients [24]. On the other hand, Hoeflich *et al*. and Rantala *et al*. studies showed an association between the MBL deficiency and increased susceptibility for infections without suffering from other immunodeficiencies [25,26].

In the present study, serum levels of IgA in Group 1 and Group 2 showed a significant decrease ($p < 0.05$) from the control group, while IgM results show a highly significant decrease ($p < 0.005$) when compared with the control group (Table 3).

There was no statistically significant difference between IgG serum levels between patients (Group 1 and 2) and control group ($p > 0.05$) (Table 3). This is in agreement with Stepensky *et al*. who detected a decrease in the three Igs levels [17]. Cohen *et al*. also stated that the participation of immunodeficiencies is weak as compared to other causes and it is remarkable that the immunodeficiency is showed only by recurrent RRIs, without association with infections of other sites [2].

B lymphocyte disorders may lead to a decrease in Igs serum levels. Siebert *et al*. in his study on B cell numbers in children having recurrent lower respiratory infections compared with control, found a significant increase in Igs synthesized cells numbers, but the median values of B cells in both groups (recurrent RRIs patients and healthy subjects) were still in the normal ranges for both age and gender [27].

Recurrent respiratory infection (RRI) when associated with IgA deficiency, a complex of primary immunodeficiency, as ataxia telangiectasia, may be considered, and a serum alpha-fetoprotein measurement, a lymphocyte phenotyping and oriented karyotyping should be achieved [28]. Moreover, inflammation in infectious disease is self-limiting the absence of antigen-specific T cells and tissue damage [29].

Raniszewska *et al*. found no significant difference in B cell numbers; only 25% of his studied

group of children had decreased CD19 cell numbers and no subjects with increased B cells. RRI is not only connected with abnormalities in neutrophils and B cells but also a defective or decreased number of T cells may lead to recurrent infections, which may include opportunistic pathogens [30]. It is clear that immunity reaches high efficacy in the 5th or 6th years of age [31]. Hence, several children with RRI may not have immunodeficiency. The cause of RRI may be the childhood itself. However, it could be a warning sign for a physician, in which the RRI is important to detect or exclude disorders associated with immunodeficiency. Early immunological assessment can allow effective Ig replacement therapy in B cell deficiency. In T cell or granulocytes disorders, antibiotic or antiviral prophylaxis should be applied [32].

Conclusion

IgA and IgM serum levels showed a statistically significant decrease in patients with genetic disorders and having recurrent respiratory infections as compared to the normal control group.

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