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APPLICATION OF EIGHT- COLOR FLOW CYTOMETRY IN THE EVALUATION OF PRIMARY IMMUNE DEFICIENCIES: EXPERIENCE FROM KING HUSSEIN MEDICAL CENTER

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ABSTRACT

Background: Primary Immunodeficiency diseases comprise a clinically and immunologically heterogeneous group of immune system disorders. Diagnosis of these disorders is supported by the use of flow cytometry which provides a rapid, sensitive and cost effective results. **Aim:** To focus on the significant applications of flow cytometry in the evaluation of Primary Immune Deficiencies. **Materials and Methods:** We revised flow cytometry analysis of 192 patients who were referred to the immunology and allergy divisions at King Hussein Medical Center based on clinical suspicion of primary immunodeficiency disease during the period from January 2016 to June 2021. **Results:** The files of 192 patients with history of persistent and recurrent infections were studied. Of these patients, the age ranged from 4 days to 12 years with a mean age of 6 years. Male to female ratio was 2:1. Primary immune deficiency was diagnosed in 90 patients (47%), of these 86.7 % were diagnosed by using flow cytometry based assays and 13.3 % the diagnosis was based on genetic mutation study. Diagnosis of severe combined immune deficiency was identified in 32.2%. There were 43.3% patients with predominant antibody deficiency (common variable immune deficiency and x- linked agammaglobulinaemia. 4.5 % patients were identified with Leukocyte Adhesion Molecules Deficiency (LAD), 4.5 % patients were found to have autoimmune lymphoproliferative syndrome and hemophagocytic lymphohistiocytosis. Post bone marrow transplantation in B- and T- cell reconstitution was the diagnosis in 2.2%. Hyper-IgM syndrome, Wiskott- Aldrich syndrome, chronic granulomatous disease and immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome were identified 13.3% of patients based on molecular analysis outside. **Conclusion**: Flow cytometry with its advances in the last years appears to be a highly sensitive and rapid tool for diagnosis and classification of primary immune deficiency, and it is essential as a first line investigation for further early evaluation to improve survival for immunodeficient patients.

KEYWORDS: Immunodeficiency, Flow cytometry, diagnosis.

INTRODUCTION

Primary immunodeficiency diseases (PID) comprise a heterogeneous group of inherited disorders of the immune system.[1] PID leads clinically to recurrent infections, autoimmune inflammatory diseases, malignancies and failure to thrive. In PID, there are more than 400 different disorders with an incidence of 1:2000 live newborns according to World Health Organization.[2] Accurate and early identification and classification of PID are mandatory for appropriate management, to reduce complications and improve the prognosis for these patients.[2,3]

The majority of PIDs characterizes by complete absence, dysregulation or dysfunction of white blood cell subpopulations, cell- surface and intracellular proteins expressions.[4] For basic diagnosis and evaluation of patients suspected of PIDs, complete clinical history with focus on family history and laboratory investigations (complete blood count, serum immunoglobulin levels, antibody titers and complement function test) are necessary. However, it's difficult to diagnose PIDs based on these measures alone because of its wide range.[5] The most recent definitive tool for diagnosis of PIDs is the genetics sequencing, however, it's expensive and takes a lot of time.[6] Flow cytometry offers a sensitive, simple, inexpensive and rapid method for multi parametric cells analysis either for confirmatory diagnosis of PIDs or narrowing the genes listed for sequencing.[7]

In this review, we study the applications of flow cytometry immunophenotyping and how its aid in the diagnosis and classification of PIDs at King Hussein Medical Center.

MATERIALS AND METHODS

In order to determine the application of flow cytometry in the diagnosis and management of PIDs, we retrospectively reviewed findings of 192 flow cytometry based results of patients with history of recurrent infection and failure to thrive between January 2016 to June 2021. We have been using FACSCanto II, three-laser, eight-color flow cytometry to analyze immunophenotyping of lymphocytes subsets by using BD Simultest ^{TM} IMK –lymphocytes Kit.

This Kit is used for enumerating the percentages of mature leukocytes subsets: T-cell (CD3 +), B-cell (CD19+), helper/ inducer T-cell (CD 3+, CD 4+), suppressor /cytotoxic T-cell (CD3+, CD8+) and natural killer NK (CD3-, CD16+, CD56+). Also we used this Kit for analysis of adhesion molecules (CD11b, CD11c, and CD18).

Fresh blood samples up to 72 hours are anticoagulated with EDTA at room temperature. Monoclonal antibody reagents were added to 50 µL of EDTA whole blood, then incubated in the dark for 30 minutes. The cells were lysed using lysing solution for 10 minutes, after that centrifuged, decanted, washed with 2ml cell wash and analyzed on BD FACSCanto II. The BD Simultest- IMK lymphocytes software can be characterized up to 30,000 cells in a single sample by analysis of forward scatter, side scatter and multicolor fluorescence. Before applying samples, we routinely run quality control for verification of our results and we use CD45 as a gating marker. Special panels for PIDs were used constituting of CD45/CD3/CD4/CD8, CD19/CD16+56. To diagnose LAD I, cells were analyzed for CD15 and for LADII the gate selected for CD18, CD11c, CD11b.

RESULTS

The records of 192 patients who presented with recurrent infections and failure to thrive were reviewed. Of these patients, the age ranged from 4 days to 12 years with a mean age of 6 years. Male to female ratio was 2:1. Ninety patients (47%) were diagnosed with PIDs, of these 86.7 % were diagnosed by using flow cytometry based assays and 13.3 % the diagnosis was based on genetic mutation study. There were 43.3% patients with predominant antibody deficiency (25 patients with x-linked agammaglobulinaemia and 14 cases with common variable immune deficiency) as shown in table 1. The diagnosis of severe combined immune deficiency (SCID) was identified in 29 patients (32.2%), of which, there were 11 patients with T (CD3) negative, B (CD19) positive and NK (CD16, CD56) negative, 4 patients with T (CD3) negative, B (CD19) positive and NK (CD16, CD56) positive, 5 patients with T (CD3) negative, B (CD19) negative and NK (CD16, CD56) negative and 9 patients with T (CD3) negative, B (CD19) negative and NK (CD16, CD56) positive.

Four patients (4.5%) were found to have Leukocyte Adhesion Molecules Deficiency (LAD), which were negative for CD11c/ CD18 and negative for CD11b/ CD 18 on the gated neutrophils. 4 patients (4.5%) were identified as autoimmune lymphoproliferative syndrome and familial hemophagocytic lymphohistiocytosis. Post bone marrow transplantation in B- and T- cell reconstitution was the diagnosis in 2 patients (2.2%) with appropriate flow cytometry immunophenotyping. On the other hand, 13.3% of patients were only diagnosed as Hyper-IgM syndrome, Wiskott- Aldrich syndrome, chronic granulomatous disease and immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome based on molecular analysis outside.

Table 1: Frequency of different types of PID diagnosed by using flow cytometry based assays at KHMC.

PID	Number of patients	%
Predominent antibody deficiency - Common variable immunodeficiency - x- linked agammagloulinemia	39 14 25	43.3
Severe combined immunodeficiency T-, B+, NK- T-, B+, NK+ T-, B-, NK- T-, B-, NK+	29 11 4 5 9	32.2
Leukocytes adhesion deficiency 1	4	4.5
AI-lymphoproliferative syndrome Familial Hemophagocytic Lymphohistiocytosis	4	4.5
Post bone marrow transplant	2	2.2

DISCUSSION

Primary immunodeficiencies (PID) are a clinically and immunophenotypically heterogenous group of inherited disorders of immune system with more than 400 different disorders resulting from genetic defects with an incidence of 1:2000 newborns. [2, 9] The major clinical

manifestation in the majority of PID is recurrent infections, failure to thrive, autoimmune diseases and malignancy disposition.[1] Although the initial suspicion of PID diagnosis is based on clinical, it is often difficult to diagnose PID according to clinical history only as the spectrum of PID is broad.[5] To confirm the diagnosis of different types PIDs, laboratory investigations such as flow cytometry and genetic studies are crucial.[10]

Flow cytometry is a routinely simple available laboratory tool to analyze cells in suspension such as peripheral blood specimens, bone marrow aspirates, cerebrospinal fluids and other tissues suspension.[4] Flow cytometry appears to provide a useful and powerful tool for diagnosis and classification of PIDs. The clinical utility of flow cytometry emerged a rapid, cost-effective and sensitive method in the initial workup, evaluation and subsequent treatment of different PIDs.[4, 7] Application of immunophenotyping in diagnosis of PIDs can be used for quantification of lymphocytes and lymphocytes subsets assay in peripheral blood, T-cell proliferation, naïve T-cell markers, determination of surface and intracellular proteins and detection of degranulation assay. These assays can be utilized as essential diagnostic method to support the clinical suspicion of PIDs.[9, 10]

We reviewed the records of 192 patients. Ninety patients (47%) were diagnosed with PIDs, of these 86.7 % were diagnosed by using flow cytometry- based assays and 13.3 % the diagnosis was based on genetic mutation study. In contrast to a previous study done in Jordan, which reported that flow cytometry-based diagnosis was recognized in 50.3% of the patients and 43.7% patients were diagnosed based on clinical and laboratory criteria.[3] Another study done by Kwon W et al, identified that 10 out of 60 patients were diagnosed by flow cytometry testing without genetic tests.[11]

In our study, we found that 43.3% of patients had predominant antibody deficiency (25 patients with x-linked agammaglobulinaemia and 14 cases with common variable immune deficiency). A previous study done in Jordan recognized that 10.3% of their patients had predominant antibody deficiency, 3 patients of them with common variable immune deficiency, who were not diagnosed by flow cytometry assay due to lack of switched memory B cells recognition in the laboratory.[3]

Flow cytometry immunophenotyping assay in our study was mainly restricted to confirm the diagnosis of B cell deficiency, SCID, LAD, autoimmune lymphoproliferative syndrome and familial hemophagocytic lymphohistiocytosis. While diagnosis of Hyper-IgM syndrome, Wiskott- Aldrich syndrome, chronic granulomatous disease and immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome was made by molecular analysis. This inadequacy of flow cytometry assessment of PIDs would make it hard to diagnose patients and expect the outcome in those who are at high risk of having PIDs.

We reviewed the files of 135 patients with recurrent or persistent infections to study the frequency of flow cytometry-based diagnosis of PID.

Positive family history of PIDs made the diagnosis of SCID at birth by flow cytometry immunophenotyping assay. SCID were encountered in 32.2% of PIDs patients, of which, the following panels identified; [T (CD3) negative, B (CD19) positive and NK (CD16, CD56) negative], [T (CD3) negative, B (CD19) positive and NK (CD16, CD56) positive], [T (CD3) negative, B (CD19) negative and NK (CD16, CD56) negative] and [T (CD3) negative, B (CD19) negative and NK (CD16, CD56) positive] which were seen in (11, 4, 5 and 9) patients respectively. Ninety percent of patients with SCID were lately diagnosed with respiratory failure which resulted in impractical and hazardous bone marrow transplantation. The decreased knowledge of PIDs and unavailability of Flow cytomerty immunophenotyping caused the late diagnosis. The variety of PIDs in which flow cytometry is confirmed to be beneficial clinically and diagnostically is considerably extended.[12, 13]

Flow cytometry immunophenotyping was useful in confirming the diagnosis of LAD cases in patients who had the same clinical findings of others, which were identified in 4.5%. Similarly, autoimmune lymphoproliferative syndrome and familial hemophagocytic lymphohistiocytosis were identified in 4.5% for each. A previous study conducted in Korea, found that 3 patients out of 60 had LAD and 2 patients for autoimmune lymphoproliferative syndrome and familial hemophagocytic lymphohistiocytosis; one for each.[11]

Post bone marrow transplantation in B- and T- cell reconstitution was the diagnosis in 2.2% of patients with appropriate flow cytometry immunophenotyping assay. A primary marker of T-cell reconstitution was established by observing the absolute CD3 count in peripheral blood 6 week post-transplant in all patients.[14] B cell reconstitution was found by B cell counting.

Seventy seven

(57%) patients were males and 58(43%) were females. They aged between 2 and 120 months with a mean age of 13 months.

CONCLUSION

Flow cytometry with its advances in the last years appears to be a highly sensitive and rapid tool for diagnosis and classification of primary immune deficiency. While genetic analysis is the specific approach to diagnose PIDs, flow cytometry is efficient to evaluate patients with PIDs at reasonably low cost, and it is essential as a first line investigation for further early

evaluation to improve survival for immunodeficient patients.

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