Immunol Genet J, Vol. 4, No. 1, Mar 2021, pp.60-66 **DOI: https://doi.org/10.18502/igj.v4i1.8395**

Original Article

Clinical Manifestations and Laboratory Findings in Patients with Leukocyte Adhesion Deficiency (LAD)

Matineh Nirouei[1], Arman Maghoul[1], Marzieh Heidarzadeh[2]*, Reihane Sharif[3]

[1] *Alborz University of Medical Sciences, Karaj, Iran*
[2] *Department of Immunology and Allergy, Kashan University of Medical Sciences, Kashan, Iran*
[3] *Health Information Management Research Center, Department of Health Information Management and Technology, School of Allied Health Professions, Kashan University of Medical Sciences, Kashan, Iran*

Received: 08 January 2021; Accepted: 14 March 2021

Abstract

Objectives: Leukocyte Adhesion Deficiency (LAD) is a rare, inherited, immunodeficiency disease which is caused by defects in the leukocyte adhesion process. The migration of leukocytes to the blood vessel's wall, needs multiple steps called adhesion cascade. In LAD, defects in rolling, integrin activation and firm adhesion of the leukocytes have been described.

Methods: In this study, we selected 67 patients with the confirmed diagnosis of LADs, from Iranian immunodeficiency registry center. A demographic information of the clinical complications and laboratory data were obtained from all the patients to evaluate the clinical manifestations.

Results: A total of 67 patients (38 male and 29 female), with a median age of 18 months old, were included in the present study. The first presentations were omphalitis in 28.35% of the cases, followed by delayed umbilical cord separation in 22.38% of the patients. The frequency of delayed umbilical cord separation was 41.8%, and was higher among other manifestations of our patients. Cellulitis and Omphalitis were observed in 40.3% and 38.8% of the patients, respectively. Regarding the laboratory findings, we found leukocytosis in 86.6 %(neutrophil dominant in 76.1%), and anemia in 77.6%, and thrombocytosis in 25.4% of the patients.

Conclusion: We indicated in the present study that the most common clinical manifestations, were delayed umbilical cord separation and recurrent infection in Iranian patients with LAD disorders. In laboratory findings, we found leukocytosis in most of the patients. CD18 was decreased in more than 90 % of the patients.

Keywords: Adhesion; Immunodeficiency; Leukocyte; Recurrent Infection

***Corresponding Author:** Marzieh Heidarzadeh, MD
5th of Qotb –e Ravandi Blvd, Kashan University of Medical Sciences, Kashan, Iran
E-mail: marz_heidar@yahoo.com

How to cite this article

Nirouei M, Maghoul A, Heidarzadeh M, Sharif R. Clinical Manifestations and Laboratory Findings in Patients with Leukocyte Adhesion Deficiency (LAD). Immunology and Genetics Journal, 2021; 4(1): 60-66.
DOI: 10.18502/igj.v4i1.8395

Introduction

Leukocyte mobilization to site of inflammation, is a vital defense mechanism against infections and tissue injuries (1). Inflammation, consists of several steps, including coordinated delivery of blood components like plasma and leukocytes. The migration of leukocytes to the site of inflammation, needs the circulation of leukocytes; and their attachment to the blood vessel's wall through multiple steps, is called adhesion cascade (2). Several families of adhesion molecules ,mediate various steps of the adhesion cascade (3). Adhesion molecules are expressed on both resting and stimulated endothelial cells and leukocytes, and any defect in these molecules, leads to the manifestation of Leukocyte Adhesion Deficiencies (LADs).

Overall, there are four distinct steps for moving leukocytes from bloodstream to the tissues, including leukocyte rolling, activation, firm adhesion and transmigration. Defect in each step of adhesion cascade, contributes to the development of LAD. Based on genetic mutation, three type of LAD (Types I, II and III) have been recognized. LAD-I is the most common form of LAD, caused by mutations in the gene *ITGB*2, encoding for the common beta 2 subunit of the integrin (CD18), located on the long arm of chromosome 21q22.3. Defects in this gene, leads to decreased expression of CD 18 molecule on leukocytes. This autosomal recessive genetic disease, results in manifestation of recurrent bacterial infections, primarily localized to skin and mucosal surfaces (4). LAD II, results from defects in expression of Sialyl Lewis X (SLeX), involved in the rolling phase. This form of LAD is extremely rare, almost less than 10 cases were reported worldwide (5). In addition to recurrent infections, these patients manifest severe mental retardation, with a very short stature and facial stigmata. LAD-III, is a result of a mutation in *Fermitin Family Homolog 3 (FERMT3)* gene. This mutation leads to absence of the kindlin-3 expression, a protein involved in the regulation of integrin activation (6). Patients with LAD3, demonstrate some clinical symptoms, such as severe recurrent infections, a bleeding tendency, and significant leukocytosis. In this study, we describe demographic, clinical manifestation and laboratory finds of the patients with LAD disorders. Few reports regarding LAD in Iranian patients, persuaded us to report these data in order to better diagnose and treat the patients.

Materials and methods

Study population

Based on data obtained from a group of LADs patients, who were referred to Iranian Immunodeficiency Registry Center at Children's Medical Center Hospital in Tehran, Iran, the clinical and laboratory data of these patients, were retrospectively evaluated. The diagnosis of LAD in the patients was based on the typical clinical presentations, including recurrent severe infections, impaired pus formation, defective wound healing, along with laboratory demonstration of leukocytosis, decrease or near absence of CD18 and its associated molecules, CD11a, CD11b, CD11c on leukocytes.

Data collection

Medical records of our patients were reviewed to collect data, such as demographic data, clinical manifestations, medical history, and laboratory findings. Then, we designed a 2-page questionnaire which included; (1) demographic information including current age, sex, age at first clinical presentation, age at diagnosis, diagnostic delay, alive/dead, consanguinity of parents; (2) clinical complications, such as recurrent infections (pneumonia, liver abscess, cutaneous abscess, meningitis, fever and seizure, lung abscess, bronchiectasis) heart failure, kidney failure, autoimmune disease, malignancy, and (3) laboratory tests' data, including the peripheral blood leukocyte counts, measurement of serum immunoglobulin levels, assessment of complement level and function and CD markers were recorded.

Statistical analysis

We used SPSS software version 22, for our statistical analysis. Furthermore, based on the findings of evaluation, the parametric and nonparametric analyses were performed. A p-value of 0.05 or less was considered as statistically significant in this study.

Results

Demographic data

A total of 67 patients (38 male and 29 female), with a median current age of 18 months old were included. The youngest patient was 1 month old, and the oldest 72 months old at the time of the study. The mean age of onset of the symptoms, was 174.6±370.68 days, and the mean age at diagnosis was 24.63±38.06 days. The median age of delayed diagnosis was1.75 months (the interquartile range: 23.25-1). The prevalence of LADs in men was more than women (56% against 43%), and the prevalence of parental consanguinity was noted in 85.1 % of the patients. 58.2% of the patients had a family history of LADs or other autoimmunity, while the remaining patients didn't have any family history of the mentioned (41.8%). 13.4 % of the patients included, died and 38.8% are still alive, whereas the mortality status of the rest 47.8% is unavailable.

Clinical manifestation

The commonest first presentation in our patients were omphalitis, which occurred in 19 patients (28.35 %), following the delayed umbilical cord separation in 15 patients (22.38 %), respiratory tract infection in 10 patients (14.92 %), and fever in 5 patients (7.46 %). Data of the first clinical manifestations are mentioned in **Figure 1**.

The frequency of delayed umbilical cord separation was 41.8%, which was higher than other manifestations in our patients. Cellulitis and omphalitis were observed in 40.3%, and 38.8% of the patients, respectively. Regarding the infectious complications, pneumonia and recurrent infection were found in 35.8% of the patients, abscess were in 31.3% of the patients, poor wound healing were seen in 29.9% of the patients, otitis were manifested in 28.4% of the patients, oral ulcers were reported in 22.4% of the patients, and diarrhea were identified in 20.9% of the patients. Mucosal infections such as gingivitis and candidiasis were observed in 20.9% and 16.4% of patients, respectively.

Among non-infectious manifestations, splenomegaly, hepatomegaly, and Failure To Thrive (FTT) were observed in 13.4%, 11.9%, and 19.4% of the patients, respectively.

Rare clinical manifestations were also observed in our patients, including sepsis in 3% of the patients, sinusitis and lymphadenopathy in 4.5% of the patients, urinary tract infection and fever in 6% of the patients and pharyngitis in 9% of our patients. Autoimmunity and malignancy were reported in none of the patients. Distribution of the clinical manifestations in our LAD patients is provided in **Figure 2**.

Laboratory data

In laboratory findings, we found leukopenia in 1 patient (1.5%) and leukocytosis in 58 patients (86.6 %). Lymphocytosis and lymphopenia were found in 61 (91%), and 6 patients (9%),

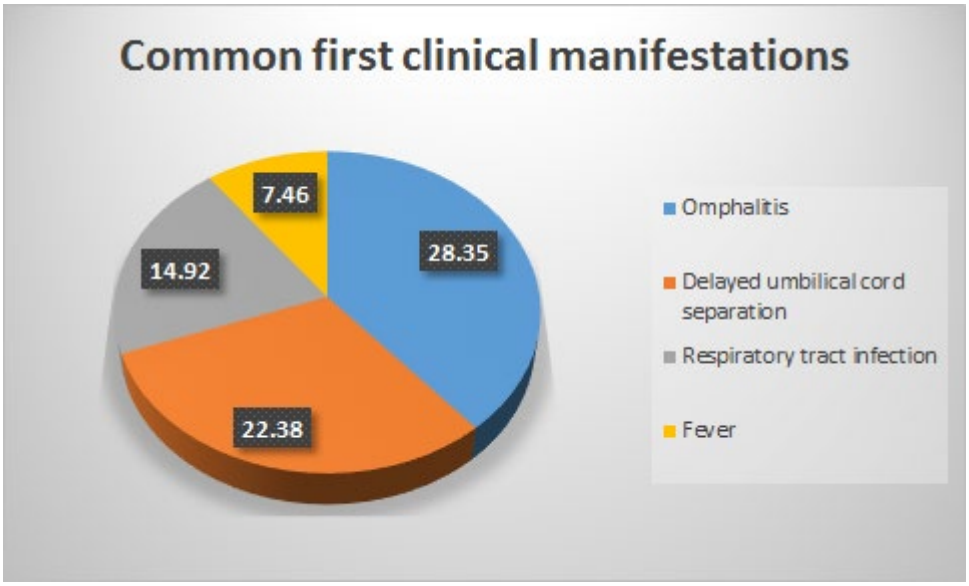

Figure 1. First clinical manifestations in LAD patients

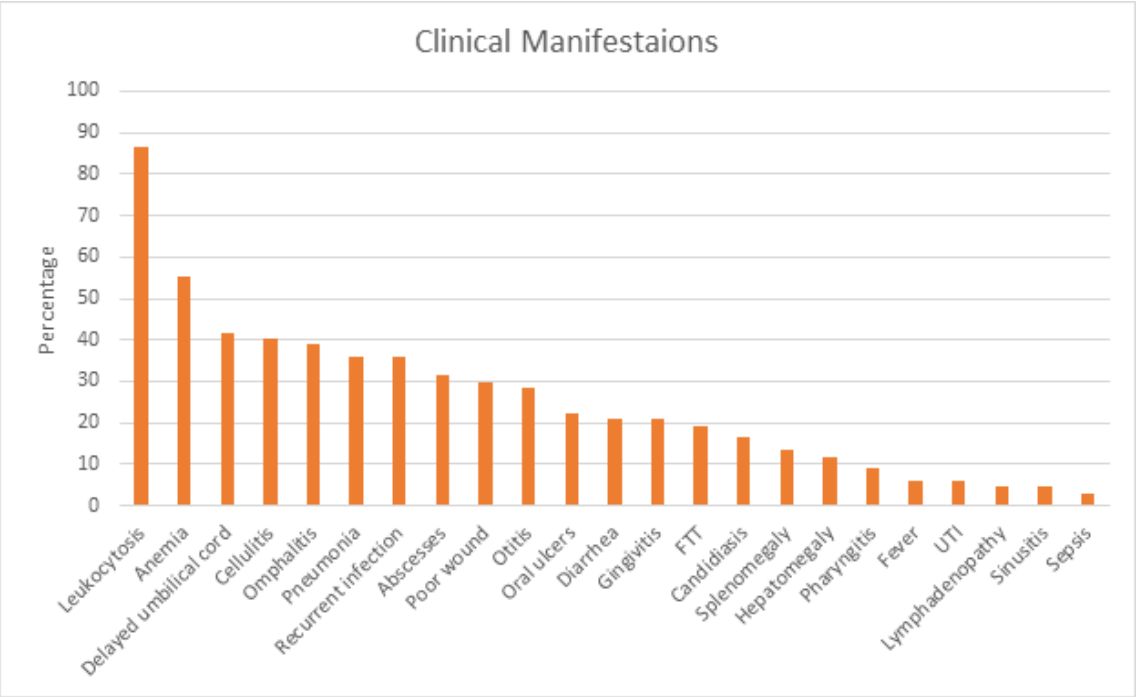

Figure 2. Clinical manifestations in LAD patients

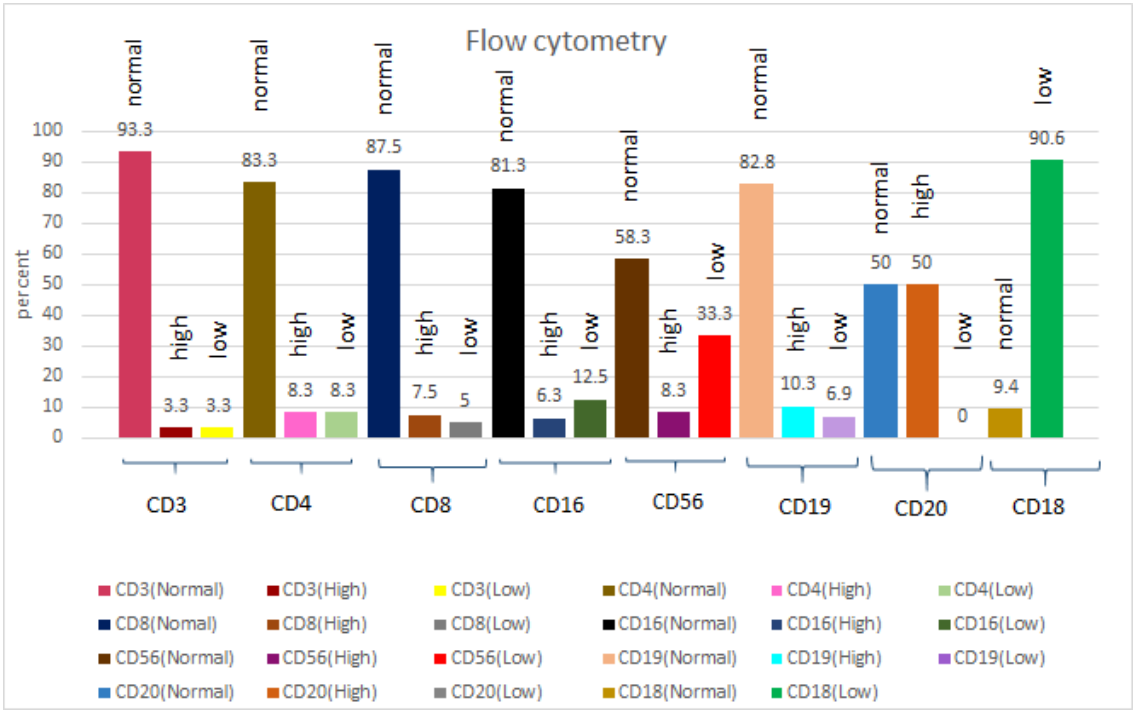

Figure 3. Abnormality in various CD markers of LAD patients

respectively. We also found neutrophilia in 51 patients (76%) and neutropenia in 16 patients (23.9%). Regarding other hematologic abnormalities, anemia was found in 52 patients (77.6%), following thrombocytopenia in 5 patients (7.5%), and thrombocytosis in 17 patients (25.4%).

Concerning to CD markers, we illustrated abnormality in various CD markers in **Figure 3**. Among CD markers, highest decrease was

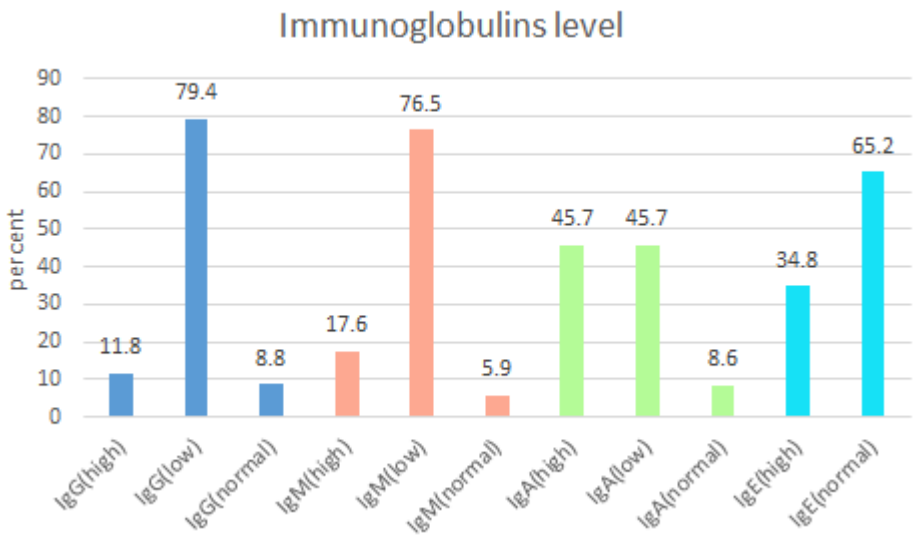

Figure 4. Abnormality in various immunoglobulin levels of LAD patients

Table 1. Immunoglobulins levels in LAD patients

Immunoglobulins level	High		Normal		Low	
	Numbers	Percentage	Numbers	Percentage	Numbers	Percentage
IgG	27	79.4%	4	11.8%	3	8.8%
IgM	26	76.5%	6	17.6%	2	5.9%
IgA	16	45.7%	16	45.7%	3	8.6%
IgE	15	65.2%	8	34.8%		

observed in expression of CD18 marker as expected. CD56+ and CD16+ cells (NK cells), had the highest reduction after CD18+ cells. CD20+ cells were the cells that did not indicate any reduction (0%). Majority of the T-cells (CD3+ cells, CD4+ cells and CD8+ cells) indicated a normal count.

Regarding the immunoglobulin levels, **Table 1** and **Figure 4** indicate normal, low and increased levels of immunoglobulins. The most reduction of immunoglobulin levels are related to IgG (79.4%) and IgM (76.5%), respectively.

Discussion

LAD is about the absence or reduced expression of the common β subunit (CD18) of the integrin receptor molecules LFA-1 and Mac-1, leading to reduced or in some cases near absent leukocyte immigration. These defects result in severe bacterial infection, and impaired wound healing as well as neutrophilia. Recurrent fungal and bacterial infections without pus formation occurred. This study describes the clinical and laboratory findings of Iranian patients with LAD. Considering the high rate of consanguineous marriages in our region, LAD is one of the most common primary immunodeficiencies diagnosed in Iran. In our study most of the patients' parents were in consanguineous marriages (85.1%). This is in concordance with the previous Iranian study published in 2007 (93.3% consanguinity) (7). However, in the study published by Madkaikar et al. in 2012, only 2 out of 7 patients had consanguine parents (8). Hence, it could be important to warn the general population how consanguinity can be a risk factor for developing LAD in the next generation.

In this study we evaluated the data of a total of 67 Iranian patients with LAD. Thirty eight cases (56.7%) were male and twenty nine cases (43.3%) were female; so we observed a male predominance in LAD patients, which is in concordance with the previous studies (7,9,10). We indicated that the majority of our patients (86.6%), had the onset of disease in the first year of life. Previous studies have also indicated the same results, as Deshpande

et al. reported the age of onset within 6 months of life, and Madkaikar et al. have reported a median age at onset of 10.5 days for their patients (9,10). These findings demonstrate that the onset of LAD is manifested in the first month or year of life, and these patients are in need of an early diagnosis and treatment.

In our study, infection was the most common manifestation of the LAD's patients. This is in concordance with the previous studies (11). The most frequent infections in our patients were cellulitis, omphalitis and pneumonia. This is the same as what had been observed in the previous studies (2,7,9). When considering only the first clinical manifestation, umbilical cord complications were the most commonly reported (Omphalitis in 36% and umbilical cord separation delay in 29% of the cases), which also complies with the previous studies (7,12). Overall, omphalitis, delayed umbilical cord separation, and the respiratory infections consisted as the most first clinical manifestations in LADs patients.

Periodontitis is a one the most important manifestation of LAD type-1. This periodontitis is an aggressive form of inflammatory bone loss, despite the lack of neutrophil functions in periodontal infection, and could lead to premature tooth loss (13). Majorana et al. and Cox et al reported a 13 year old boy with LAD–1 and severe periodontitis (14), and a 3 year old girl with gingivitis (15), respectively. These cases showed that periodontitis could cause premature tooth loss, hence it is a very important clinical manifestation among LAD patients.

Regarding mucosal clinical manifestations, Moutsopoulos et.al. reported clinically diagnosed periodontitis with severe loss of tooth-supporting connective tissue and bone in all of their patients (5 cases) in their LAD-I cohort (16). However, only 20.9% of the patients in our study had gingivitis; this could be because of our relatively larger study population.

Uzel et al. in 2016, reported 3 patients with LAD, and all 3 patients suffered from inflammatory bowel disease. Whereas, in our patients, no inflammatory bowel disease was reported, this could be because we didn't have the contact information, and the fact that the inflammatory bowel disease could occur in the next years of life of our patients (17). Akbari et al. reported an 11 months old patient with gangrenous perianal wounds, and they have debridement of wounds and colostomy, and M Madkaikar et al reported 7 patients with Necrotic skin lesions, although in our patients there were no signs of gangrenous wound, and it could be due to the rareness of gangrenous wound manifestation of LAD (8,18).

The beta2 integrin is only expressed on leukocytes. Decreased expression of the common b2 subunit, leads to a similar decrease in the expression of all four subunits on the leukocyte surface, resulting in decreased CD18 expression. In concordance with the previous studies, we found that 90.6% of the patients had lower CD18 expression than the normal range (4,12). Leukocytosis, in particular neutrophilia, is one of the characteristic features of the LADs. Patients with LAD I, show mild to moderate neutrophilia in the absence of overt infection (2). In our study, leukocytosis was seen in 86.6%, and neutrophil dominance in 76% of the cases. Movahedi et al. (15 cases) and Madkaikar et al. reported leukocytosis in all of their patients (30 cases) (7,10). These findings indicates that Low CD18 expression and leukocytosis, especially neurophilia, are the typical laboratory findings in LAD patients.

The objective of this study was to evaluate the clinical presentation and laboratory findings in a long-term course in patients with LADs. We faced some limitations during this retrospective study. The data time span was over 10 years, and there was missing data in some of the forms. We didn't have contact information for all of the patients, so we couldn't check on the patients for evaluating their current status (e.g., being alive).

Conclusion

In this study, we have described clinical and laboratory features of Iranian patients with LAD. The common first presentations were omphalitis, and then were the delayed umbilical cord separation. The most common clinical manifestations, were delayed umbilical cord separation and recurrent infection. In the laboratory findings, we found leukocytosis in most of the patients. CD18 was decreased in more than 90 % of the patients, resulting in defective leukocyte adhesion and increase leukocyte in the blood stream, and subsequently the existence of more recurrent infections and opportunistic

infections in these patients. In addition, the present study indicates LAD as one of the most common primary immunodeficiencies diagnosed in Iran, which could be due to the high rate of consanguineous marriages in our region.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by "Research Center for Immunodeficiency". We thank all the patients who have helped us to do this research.

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