# Immunophenotyping By Cluster Of Differentiation (CD) Markers In Patients With Atopic Dermatitis

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## Abstract

Determination of concentrations of cluster of differentiation (CD) markers in patients with atopic dermatitis and comparison with healthy individuals were carried out in this study.

It has been found that the mean of concentrations reached (82.2, 55.7, 28.7 and 21.9) % of +ve lymphocyte for AD patients and (72.2, 40.3, 18.5 and 13.1) % of +ve lymphocyte for healthy individuals of CD3, CD4, CD8, and CD19 respectively. We found that means of CDs had highly values than those of healthy individuals in (65.7%, 75.9%, 94.4% and 68.5%) of AD patients for above CDs respectively. (P< 0.05)

## INTRODUCTION

Atopic dermatitis (AD) was first introduced in 1933 by Hill and Sulzberger in recognition of this close association between AD and respiratory allergy(1). The concept that AD has an immunologic basis is supported by the observation that patients with primary T-cell immunodeficiency disorders are frequently associated with elevated serum IgE levels and eczematoid skin lesions indistinguishable from AD(2). Cluster designation of monoclonal antibodies (cluster of differentiation (CD) designated from 1<sup>st</sup> to 8<sup>th</sup> workshops on international human leukocyte differentiation antigens with total number of (247) CDs(3). Leukocytes express distinct assortments of molecules on their cell surfaces, many of which reflect either different stages of their lineagespecific differentiation or different states of activation or inactivation. Leukocyte cell surface molecules are routinely detected with anti- leukocyte monoclonal antibodies (mAbs). Using different combination of mAbs, it is possible to chart the cell surface immunophenotypes of different leukocyte subpopulations, including the functionally distinct mature lymphocyte subpopulations of B-cells, helper T-ells (TH), cytotoxic T-cells (Tc), and Natural Killer (NK) cells(3). The dermal cellular infiltrate in AD mainly consists of  $CD_4^{+}$  and  $CD_8^{+}$  T-cells with a  $CD_4/CD_8$  ratio similar to peripheral blood levels(4). In recent studies, CD<sub>8</sub> <sup>+</sup> CLA<sup>+</sup> T-cells in induction of immunoglobulin E (IgE) and prolonged eosinophil survival(<sub>5</sub>).

The aim of the present study is evaluate/ or determine immunophenotyping of AD patients by measuring concentration of clusters of differentiation (CDs) in patients suffering from atopic dermatitis.

## MATERIALS AND METHODS

5 mls of venous blood were collected by aseptic venipuncture from (108) AD patients and the control group (100 healthy persons) in sterile glass tubes containing 10 IU/ml of sodium heparin or EDTA (anticoagulated tubes). All steps of CD marker procedure were carried according to instructions of supplied company (Bio source Int., Belgium)(<sub>677</sub>), that include three main steps:

1. Isolation of mononuclear (MN) cells (lymphocytes) by density gradient centrifugation using lymphoprep to give a lymphocyte concentration of  $1-2 \times 10^7$  cell/ml.

2. Measuring the lymphocyte count and viability by trypan blue exclusion test. More than 95% of lymphocyte viability was ensured to perform the immunophenotyping analysis(8).

3. Determine the specificity and concentration of known fluorescent monoclonal antibodies (McAbs) by immunoflorescence technique.

We used four McAbs in the present study :

Anti-CD<sub>3</sub>-McAb Anti-CD<sub>4</sub>-McAb Anti-CD<sub>8</sub>-McAb Anti-

#### CD<sub>19</sub>-McAb

A reaction was considered positive when cells have multiple fluorescent dots on the membrane or homogenous bright green membrane fluorescence( $_7$ ).

## STATISTICAL ANALYSIS

Chi-square test and ANOVA test were carried by using SPSS program ver.11.

## RESULTS

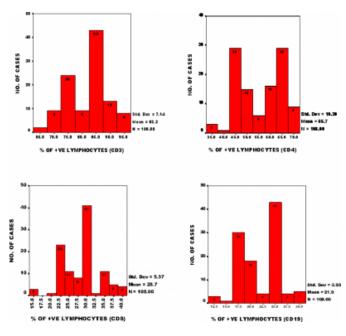
The mean of CDs concentration (CD<sub>3</sub>, CD<sub>4</sub>, CD<sub>8</sub> and CD<sub>19</sub>) of AD patients are (82.2, 55.7, 28.7 and 21.9) % of + ve lymphocytes respectively while the means of CDs concentration of healthy / or control group are (74.2, 40.3, 18.5 and 13.1) % of +ve lymphocytes of above CDs respectively. Fig (1)

The concentration of CDs (CD3, CD4, CD8 and CD19) had a highly values or means than those for healthy/ or control group in (65.7%, 75.9%, 94.4% and 68.5%) of AD patient respectively with significant differences (P< 0.05).

The statistical similarity analysis of CDs illustrated that CD3, CD4 and CD8 had similarity ranged between 94-97%, and CD19 splitted from its in similarity ratio near 63.86%. Fig (2).

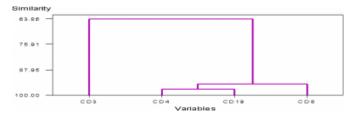
#### Figure 1

Figure 1: Illustrated all of studied clusters of designation ( CD ): CD3 , CD4 , CD8 and CD19 for AD patients. ( p < 0.001 )



#### Figure 2

Figure 2: Statistical similarities between various types of clusters of differentiation ( CD ) .( p< 0.001)



#### DISCUSSION

Our results showed a significant elevation of various types of studied CDs value, those results were compatible and confirmed by the results of other studies that evidenced correlation between various CDs and atopic dermatitis( $_{9,10,11,12}$ ). While, there are many other studies interested in other CDs such as CD23, CD24, CD26, CD28, CD30, CD45,CD83, CD137 and CD153( $_{13,14,15,16,17}$ ).

The importance of study CDs and their reactions with atopic dermatitis is interrupt with CD functions and their affecting role in immunopathology of allergic and /or atopic diseases(<sub>3</sub>). CD3 was associated with T-cell antigen receptor. Required for cell surface expression of and signal transduction by TCR, cytoplasmic domains contain ITAM motifs and bind Cytoplasmic tyrosine kinase(<sub>18</sub>). CD4 is coreceptor for MHC class II molecules. Bind Lek on cytoplasmic face of membrane. Receptors for HIV-1 and HIV-2(<sub>19</sub>). CD8 is coreceptor for MHC class I molecules. Binds Lek on cytoplasmic face of membrane(<sub>20</sub>). And CD19 forms complex with CD21 (CR2) and CD81 (TAPA-1), coreceptor for B-cells-cytoplasmic domin. Binds cytoplasmic tyrosine kinases and PI-3 kinase(<sub>21</sub>).

### CONCLUSION

We conclude that there is an elevation of concentrations of all studied CDs of patients with atopic dermatitis in comparison with control / or healthy individuals.

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