INTRODUCTION

Organic acidemias are group of disorders characterized by increased excretion of organic acids in urine. They result primarily from deficiencies of specific enzymes in the breakdown pathways of amino acids or from enzyme deficiencies in beta oxidation of fatty acids or carbohydrate metabolism ***(Zamani, 2012).***

Patients with organic acidemia suffer from recurrent infections with high morbidity and mortality. Some researchers have reported patients with organic acidemia with recurrent unusual infections ***(Nakamura and Tokura, 2010).***

Propionic acidemia is characterized by tissue accumulation of propionic acid. Propionic acid inhibits a mitogen-activated proliferation of human peripheral lymphocytes in vitro. Methyl-malonic acid may have similar immunosuppressive effect as propionic acid, which leads to recurrent infections ***(Wajner et al., 1999).***

Good knowledge of probable defects in immune system can lead to administration of suitable broad spectrum antibiotics to reduce morbidity and mortality ***(Najjarbashi et al., 2015).***

Aim of the Work

This study aims to assess the number and function of T lymphocytes and NK cells in patients with organic acidemia (methyl-malonic or propionic acidemias) and the relation of their immunological parameters to the organic disease control.

THE IMMUNE SYSTEM

Immunity is the complex of cellular and/or humoral events following the entry of foreign substances into the host. Two general systems of immunity with specialized roles in defending against infection have been selected during evolution ***(Del Rio et al., 2004)***.

Innate immunity:

Innate immunity provides the early lines of defense against microbes. It is void of both fine tuned discrimination of non-self substances and increased activity following repeated encounters, thereby demonstrating that it does not possess memory. It includes: (1) phagocytic cells (neutrophils, macrophages) and natural killer cells (NK); (2) blood proteins, including members of the complement system and other mediators of inflammation; and (3) proteins called cytokines that regulate and coordinate many of the activities of the cells of innate immunity ***(Mills et al., 2003).***

Adaptive immunity:

It becomes involved when the first level of defense fails to fully prevent infection, exemplifying a recent evaluative process, characterized by a particular specificity for offending antigens and by memory. Unlike innate immunity, it is elicited or stimulated by exposure to intruders that escaped early elimination by the innate immune system and it is armed with a versatile discriminating capacity which is potentiated by successive encountering with such agents ***(Mills et al., 2003).***

Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Specialized cells, the antigen-presenting cells (APCs), display the antigen to lymphocytes and collaborate with them in the response to the antigen ***(Staros, 2005).***

Humoral-mediated immunity:

It is the principal defense mechanism against extracellular, encapsulated microbes, their toxins and some viruses as enterovirus because secreted antibodies can bind to these microbes and toxins and assist in their elimination ***(Mills et al., 2003).***

Humoral immunity is passively transferable by serum or plasma, being mediated by antibodies with a specific attitude for reacting with the configurations responsible for its production ***(Mills et al., 2003).***

Cell mediated immunity (CMI):

It is active in the defense against intracellular microbes, such as viruses, fungi and some bacteria that survive and proliferate inside phagocytes and other host cells) mycobacterium tuberculosis and listeria (where they are inaccessible to circulating antibodies). Defense against such infections is a function of cell-mediated immunity, which promotes the destruction of microbes residing in phagocytes or the killing of infected cells to eliminate reservoirs of infection ***(Royer and Reinherz, 2004)***.

T-LYMPHOCYTES

T-cells are derived to ensure a high specificity in the critical phase of immune response to pathogens of various sources, where T cells respond to antigens only when encountered inside or on the target cell surface ***(Williamson et al., 2005).***

T- Lymphocyte development:

Like any type of blood cell, lymphocytes originate from pluripotent hematopoietic stem cells (HSC) located in the bone marrow, especially in the pelvis and iliac crest. These cells can divide asymmetrically: the daughter cell is the replication of the parent cell, while the parent cell keeps the capacity to generate more daughter cells without differentiation, the daughter cell will differentiate into the desired cell type ***(Dzierzak and Speck, 2008).*** Then, T cells development takes place nearly exclusively in the thymus. At an undetermined stage, a very early T cell precursor leaves the bone marrow, enters the blood circulation, reaches the thymus and will be called thymocytes. Thymic T cell differentiation is driven by thymic stromal cell and the factors they are secreting (cytokines and growth factors) ***(Belizário et al., 2016)*** **(Figure 1)**.

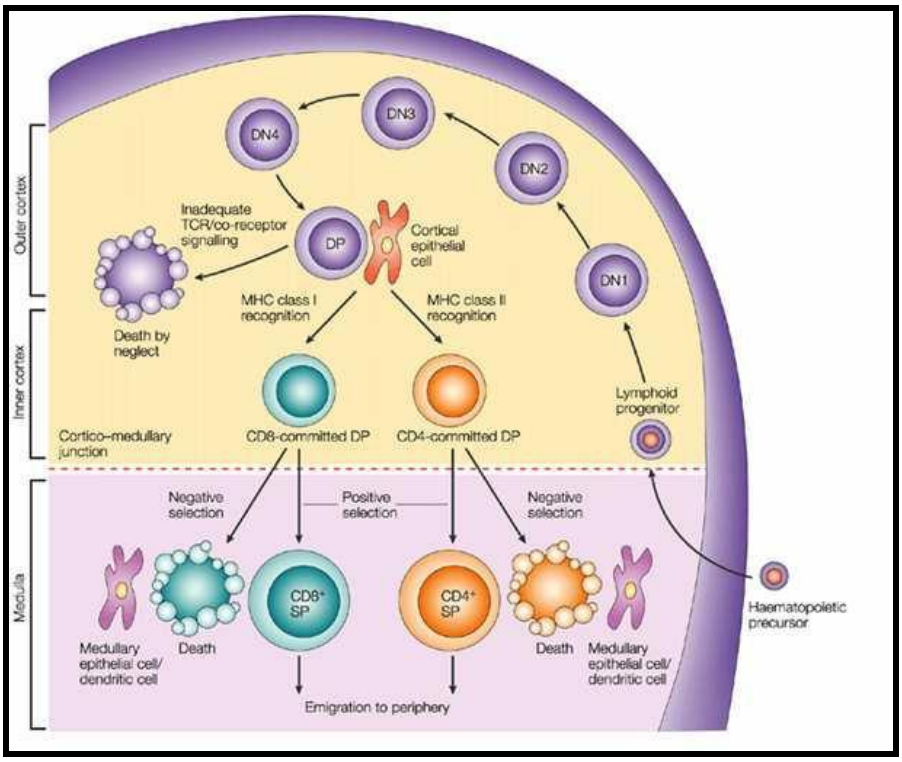

Figure (1): Overall scheme of T-cell development in the thymus *(Germain, 2002)*.

CD: cluster of differentiation; DN: double negative; DP: double positive; MHC: major histocompatibility complex; SP: single positive; TCR: T cell receptor.

T-cell Subsets:

(A) Naïve T cells:

Naïve T cells reside in secondary lymphoid tissues, such as spleen, lymph nodes and Peyer's patches in the intestine. Naive T-cells rely upon the migration of dendritic cells into the lymphoid tissues to bring information about new immunological events occurring in peripheral tissues into the appropriate areas; within secondary lymphoid tissue, the naïve

T-cells can scan the new information and possibly be activated ***(Jenkins et al., 2001).***

Since naïve T-cells continually recirculate through the blood, spleen, lymph nodes and other tissues, they are able to sample antigens derived from a wide range of distinct anatomical sites in search for a complementary peptide MHC complex. Following engagement of the appropriate complementary complex, a subpopulation of effector cells is activated and redirected to circulate between the blood and peripheral tissues. This permits effector T-cells to reach site of infection ***(Sallusto et al., 2000).***

Naive T cells circulating in the blood, express L-selectin (CD62L), chemokine receptor 7 (CCR7) and leukocyte function antigen-1 (the αLβ2 integrin LFA-1). These mediate the rolling, adhesion, and extravasation of the cells through the high endothelial venules (specialized venules found in lymphoid tissues) in peripheral lymph nodes and mucosal lymphoid organs ***(Reinhardt et al., 2001)***.

(B) Helper T cells:

Following recognition of foreign antigens, the antigen-presenting cell (APC) is activated and will migrate to the secondary lymphoid organs (SLO). When naïve T cell are activated by the antigen-presenting cell (APC), they acquire effector functions while differentiating into T helper cells (TH1, TH2, TH3, TH9 and TH17), follicular helper T cell

(TFH), regulatory T cell (Treg) or cytotoxic T cells ***(Iezzi et al., 2001)*** **(Figure 2).**

T helper cells (TH) participate in the regulation, enhancement, and recruitment of innate cells such as macrophages, neutrophils, mast cells and monocytes ***(Zhu et al., 2010).***

TH1 cells produce interferon gamma (IFNγ) and are associated with cell-mediated immune responses against intracellular pathogens; while TH2 cells produce interleukins (IL4, IL5, IL13, and IL10) and are thought to drive humoral immune responses against parasites ***(Zhu et al., 2010, Perrigoue et al., 2009, Wilson et al., 2009)*** **(Figure 2).**

The main effector cells for TH1 immunity are macrophages, cytotoxic T lymphocytes (CTL), immunoglobulin G (IgG B cell) and IFNγ producing CD4 T cell; while the main effector cells for TH2 immunity are eosinophils, basophils, mast cells, immunoglobulin E (IgE B cells), and IL4/IL5 producing CD4 T cells ***(Wilson et al., 2009).***

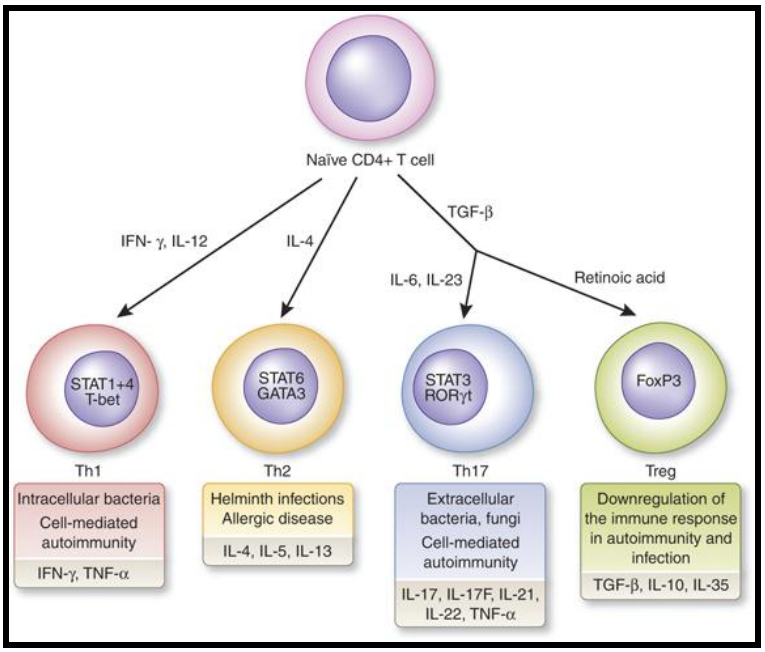

Figure (2): Differentiation of CD4+ T-cell subsets
(Jan-Eric et al., 2010).

CD: cluster for differentiation; FoxP3: forkhead box P3; GATA: Globin transcription factor; IFN: interferon; IL: interleukin; RORγt: related orphan receptor *gamma t*; STAT: Signal transducer and activator of transcription; Th: T helper; TGF: tumor growth factor; TNF-α: tumor necrotic factor α.

(C) Cytotoxic T-cells:

It is a direct attack cell that is capable of killing micro-organisms, even some of the body's own cells so they are called killer cells. The protein receptors of the cytotoxic cells cause them to bind tightly to those organisms or cells that contain appropriate binding specific antigen. After binding, the cytotoxic T-cell secretes hole-forming proteins called perforins that punch large round holes in the membrane of attacked cells.

Then fluid flows rapidly into the cell from interstitial space in addition the cytotoxic cell releases cytotoxic substances directly into the attacked cell. Almost immediately the attacked cell becomes greatly swollen and it usually dissolves shortly thereafter ***(Guyton and Hall, 2006)***.

In addition to possessing CD3, cytotoxic T-cells are also positive for the surface molecule CD8. Naïve cytotoxic cells are activated when their T-cell receptor (TCR) strongly interacts with a peptide-bound major histocompatibility complex (MHC class I molecule) to be activated ***(Edger and Sewell, 2005)***.

(D) Suppressor T-cells:

They are capable of suppressing the functions of both cytotoxic and helper T-cells. These suppressor functions serve the purpose of regulating the activities of other cells and keeping them from causing excessive immune reactions that might be severely damaging to the body's own tissues. For this reason, the suppressor cells are classified as regulatory T-cells ***(McHugh and Shevach, 2002)***.

(E) Memory T cells:

Memory T cells are antigen-experienced, long-lived T cells that are different from naïve cells in numbers and functions. They can mediate protection by mounting a faster and stronger immune response to subsequent encounters with

the invader. More than 90% of responding cells die after infection while for the 10% surviving, IL7, IL12 and an IL21/IL10/Signal transducer and activator of transcription 3 (STAT3) pathway seems to play a decisive role in their differentiation /maturation/ maintenance, at least in the CD8+ memory T cells ***(Cui et al., 2009, Cui et al., 2011).***

Memory T cells may be divided into three subpopulations based on their homing capacity, namely central memory cells (TCM), effector memory cells (TEM), and tissue-resident memory cells (TRM**) *(Mueller et al., 2013)***. While most memory cells are left behind following the massive apoptosis of effector T cells, a significant proportion (easily detectable in blood) remains after an immune response. TCM express chemokine receptor type 7 (CCR7) and CD62L (L-selectin) as well as secrete IL2 however lack the capacity to produce IFNγ and IL4. Because TCM display higher self-renewal capacity, they are associated with a memory stem cells capacity -that still need a consensus, and regarded as superior to TEM. On the other hand, TEM do not express CCR7 or CD62L, are less proliferative, but produce higher levels of interferon (IFN) and IL4. The CD4+ and CD8+ TCM mainly reside in secondary lymphoid organs, while TEM can be found in peripheral compartment ***(Reinhardt et al., 2001)***. After infection, populations of memory T cells can also reside in peripheral tissues, and recently designated as tissue-resident memory T cells (TRM) expressing CD103 and CD69 molecules ***(Masopust et al., 2010).***

(F)Regulatory T-cells:

The regulatory T-cells (Treg), limits and suppresses the immune system, and may control aberrant immune responses to self-antigens; an important mechanism in controlling the development of autoimmune diseases ***(Buckley, 2016).***

Regulatory T-cells are distinguished by the presence of the transcriptional regulator, forkhead box protein 3 (FOXP3), which appears to be a master switch gene for the development and function of regulatory T-cells. These cells also express CD25 (IL2 receptor). CD4+CD25+FOXP3+T-reg cells are believed to be important in development of allergic disease. They have been also implicated in prevention of graft rejection and graft versus host disease (GVHD) ***(Fontenot et al., 2003).***

Surface markers of T lymphocytes;

T cells and their subsets can be identified by differential expression of cell surface markers including CD3, CD4, CD8, CD25, CD127, and CD196 (CCR6). Adding markers such as CD197 (CCR7), CD62L, CD69, and CD45RO to an analysis provides important information about the potential for cells to home and localize within the body, as well as the activation status of the T-cell subset of interest. This information can also be used to identify different memory subsets ***(Mahnke et al., 2013)*** **(table 1).** Two commonly used CD molecules are CD4 and CD8, which are, in general, used as markers for helper and

cytotoxic T cells, respectively. These molecules are defined in combination with CD3+ ***(Byers et al., 1998)*** **(table 2).**

Table (1): Expression of T cell markers and their purpose.

Marker	Purpose
CD3	T cell marker
CD4	T helper cell subset
CD8	T cytotoxic cell subset
CD25	Regulatory T cell marker
CD127	Regulatory T cell marker
CD196 (CCR6)	TH17 cell marker
CD197 (CCR7)	Naïve/Memory T cell
CD62L	Secondary lymphoid tissues homing
CD69	T cell proliferation
CD45RO	Memory T cell
CD38	Calcium flux/signal transduction
CD27	Costimulation/memory
CD25	Regulatory T cell/Activation

CD: Cluster of differentiation; CCR: chemokine receptor; TH: T helper

(Mahnke et al., 2013)

Table (2): Characteristics of CD3, CD4 and CD8 markers.

	CD3	CD4	CD8
Expression and function	When antigen binds to the T-cell receptor, it transduces the activating signals.	It is expressed on normal peripheral blood lymphocytes and also expressed in low density on the cell surface of monocytes and in cytoplasm of macrophages. CD4 cells play an important role in the maintenance of immunologic tolerance to self and foreign antigen.	It is seen on suppressor cells, cytotoxic cells and some natural killer cells. CD8 cells play an important role in the maintenance of immunologic tolerance to self and foreign antigen.
Conditions increased in	The CD3 surface proteins are integral components of the functional T-cell receptor complex and increased when there is general expansion of T lymphocytes.	Cells expressing CD4 are increased in autoimmunity, allergy and when there is expansion of the T cell population.	CD8 positive cells are increased in a variety of situations such as viral infections and when there is general expansion of T lymphocytes.
Conditions decreased in	They are decreased in general failure of T cells as in HIV infections and primary immunodeficiency.	They are reduced in general failure of T cells and selectively in HIV infections and some primary immunodeficiencies.	They are reduced in many autoimmune conditions such as multiple sclerosis, thyroiditis and SLE as well as lymphocyte aplasia.

CD: cluster of differentiation; HIV: human immunodeficiency virus; SLE: systemic lupus erythematosus.

(Byers et al., 1998; Peterson and Koretzky, 1999)

The T-cell receptor for antigen:

All T–cells express a receptor for antigen that is formed by two polymorphic polypeptides linked to each other via disulfide bonds and is associated with a collection of invariant proteins called CD3. The two polypeptides that form the T -cell receptor on most cells are termed α and β, whereas a small subset of T cells have different polypeptides called γ and δ ***(Kang and Raulet, 1997).***

The polypeptides of the T-cell receptor have a diversity that is comparable to that estimated for immunoglobulin molecules. However, unlike immunoglobulins, the T-cell receptors recognize small fragments of antigen, usually peptides that are presented by major histocompatibility complex (MHC) molecules of another cell (the antigen presenting cells) ***(Kipps, 2001).***

Function of T lymphocytes:

- T cells including memory and effector cells play an important role in immunity against pathogens. Once an antigen-presenting cell presents to pathogenic naïve T cell pathogenic antigen, T cells become activated, increase in cell number and differentiate into effector cells which migrate to the site of infection and eliminate the pathogen. Effector T cells are short lived cells, while the subset of memory cells is formed with long-lived survival. Memory cells can be located in the secondary lymphoid organs

(central memory cells) or in the recently infected tissues (effector memory cells). During re-exposure to antigen during the second immune response, memory T cells undergo fast expansion and cause more effective and faster immune response versus the primary immune response, eliminating infection ***(Rosenblum et al., 2016)***.

- T helper cells (TH) play an important role in the regulation and enhancement of innate cells such as macrophages, neutrophils, mast cells and monocytes ***(Zhu et al., 2010)***.

Th1 cells produce INFγ and are associated with cell mediated immune responses against intracellular pathogens, while TH2 cells produce interleukins (IL4, IL5, IL13 and IL10) and are thought to drive humoral responses against parasites ***(Zhu et al., 2010; Perrigoue et al., 2009; Wilson et al., 2009)***.

- Cytotoxic T cells called killer cells because they are capable of killing micro-organisms, even some of body's own cells. The attacked cells become swollen and dissolves shortly thereafter ***(Guyton and Hall, 2006).***

- Regulatory T cells limits and suppresses the immune system and control aberrant immune responses to self-antigens, an important mechanism in controlling the development of autoimmune diseases ***(Buckley, 2016).***

- CD4+ T cells may also provide help during the post-priming phase that occurs at tumor site. An optimal CD4+ T-cell