

The T helper (Th) T Cytotoxic (Tc) Ratio in Hemophilia Disease

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Abstract

Background and Objectives:

In this study, we investigated the T helper (Th) to T cytotoxic (Tc) ratio in children suffered from hemophilia disease. 80 diagnostic subjects referred to Ali Asghar hospital of Zahedan city, Zahedan-Iran, were selected and the ratio of these cells were analyzed by analytic descriptive methods.

Method of study:

Two mls of EDTA anti coagulated whole blood was collected. Immunophenotyping of lymphocytes count was carried out by FACS analysis using a double CD4 and CD8 kit. The mean \pm SD of absolute numbers of CD4 and CD8 lymphocytes/ml was calculated and the ratio of CD4/CD8 was evaluated by statistical method.

Results:

We found 100% of patients were type A hemophilia. Among of them 66 (82.5%) were male. The mean age was 15 ± 3.51 years. 12 (15%) of them had mild disease intensity and 68 (85%) had sever disease intensity. The ratio of CD4 and CD8 was obtained between 0.45 and 1.44 with mean 1.79 ± 0.78 and correlation with the gender and disease intesivity was 0.095 and 0.019 respectively.

Conclusions:

The results showed that the ratio of T helper (Th) to T cytotoxic (Tc) has significant correlation with

Introduction:

Hemophilia disease is one of the X linked hemorrhagic disorders that encodes coagulation factors caused by either functional deficiency or lack of the human coagulation activity factor as a result of gene heterogeneous mutations (1). Hemophilia patients due to the deficiencies in the amount of factors are suffering from abnormal bleeding time and this leads them to have bleeding in small injuries (2). Patients with this conditions have prolonged bleeding following trauma such as injury, surgery, or having a tooth pulled. On the other hand in severe cases of hemophilia, continuous bleeding happens after minor trauma. This condition may happen in the absence of injury (spontaneous bleeding). Serious complications can be seen from bleeding into the all part of body especially in to the joints, muscles, brain, or other internal tissues (3). The disease is classified as mild, moderate, and severe based on the degree of deficiency of the coagulation factors. A few studies have reported on race ethnicity differences in hemophiliac epidemiology. The prevalence of hemophilia for 106 countries has been reported between 1998 and 2006. In American population to be 13.2 per 100000 males among white Americans, 11.0 among African-Americans, 11.5 among Hispanic males, and 4.3 among Asian/Pacific Islanders in the United States (4). In Iran based on variety factors such as molecular studies, clinical presentations, kind of treatment, development and management of patients with inhibitor, the prevalence of disease is varied.

According to the report of Dorgalaleh A Iran has the ninth largest hemophilia population in the world (5). In report of epidemiology of hereditary coagulation bleeding (HBD) disorders from Southern Iran factor VIII deficiency was the most prevalent type (50.4%) of HBD, and combined Von-Willebrand and factor XIII deficiency (2.3%) was the most prevalent type of combined factor deficiency (6). Despite of VIII frequency deficiency is common in Iran, other inherited coagulation factor deficiencies such as FXIII deficiency (FXIII D) as rare bleeding disorder inherited is not rare because of consanguineous marriage is popular, so that one-third of global FXIII D patients are located in this country (7, 8). The complex nature of diseases are included as gene mutations, uncontrolled and recurrent spontaneous bleeding, high cost of treatment, absence from school, suffer from viral diseases caused by the consumption of blood products, psychosocial problems and negative effects on patients and their families quality life (9). Two types of hemophilia have been identified as type A and Type B. Type A (also known as classic hemophilia or factor VIII deficiency) is common than type B. Boys are mostly affected by disease and girls are the gene carriers. Hemophilia A is the most common blood disorders a glycoprotein factor eight deficiency or dysfunction, that operates as a cofactor in the activation of factor X (FX) via activated factor IX (FIX), does not allow the formation of clot at the site of tissue injury. The disease intensity is related to functional deficiency of 8 factor. This disease is divided in to three major groups as severe (less than 1% factor level 8), medium (between 1 to 5%) and mild (5 to 40%) groups 26 and 31 percent of patients, it is. These groups constitute 43, 26 and 31% of patients respectively (1). Hemophilia B (also known as Christmas disease or factor IX deficiency) as X-linked recessive hereditary disorders caused by either functional deficiency or lack of the human coagulation factor 9 (hFIX). Its prevalence in men has been estimated as one in thirty thousand. The importance of disease is because as patients die due to frequently excessive bleeding, therefore their life is under threatened (10). Various mechanisms are involved in disease susceptibility such as ethnicity, family history, and lack of formation of inhibitors, FVIII gene mutations and processes that involve immune system genes. Among of them immune response to the gene may have a critical role in induction of disease. Immune responses in patients with hemophilia A are directed against clotting factor FVIII (FVIII) and are very complicated treatments. This response can manifest in congenital hemophilia as well as acquired hemophilia. The role of B Cells in breaking and maintaining tolerance to clotting factor VIII in congenital and acquired hemophilia A has been suggested by Amanda M. Actor (11) but the role of cell mediated immunity (CMI) in hemophilia patients remains to be explained. Regarding to this study, one of the component of CMI is the function of T-lymphocytes. Immunophenotyping of these cells was carried out by FACS (Fluorescent Antibody Cell Sorter) method for enumerating the absolute CD4, CD8, and CD3 T cell counts (12). CD4 play critical roles in the maintenance of immune system and achieving a regulated effective immune response to pathogens. These cells are activated after interaction with antigen proceed and presented by APCs in association with MHC – class II complex and differentiate into specific subtypes based on the cytokine production called as Th1, Th2, Th3, Th9 and Th17 each with a characteristic cytokine profile (13, 14).

CD8 or CTLs play an important role in human cellular and tumor immunity as well as lysis of autologous platelets. Platelet lysis mediated by CD8 + T cells has been demonstrated to be involved in ITP pathogenesis. These cells can also kill tumor cells, the cells infected with bacteria, viruses by

programming the cells to undergo apoptosis. (13). At the Italian Vicenza Consensus Conference in October 2007, the experts of the ITP International Working Group decided to use the term “immune” in place of “idiopathic” to emphasize the immune-mediated mechanism of the disease. They also showed that the direct dissolution of antigen-specific antibodies mediates platelet destruction and that cytotoxic T cells (CTL) increase platelet destruction. Thus they concluded that T cell immune abnormalities play crucial roles in ITP pathogenesis. These T cell abnormalities are characterized by the excessive activation and proliferation of platelet auto-antigen-reactive CTLs, abnormal numbers and functions of T regulatory cells (Tregs), production of abnormal helper T (Th) cells, megakaryocyte maturation abnormalities, abnormal T cell anergy and other factors(15, 13). A study was carried out by Hu et al in 2007. They showed that FVIII-specific CD4(+) T cells are common in hemophilia A patients by producing IFN gamma, IL-4- and TGF-beta1 suggesting role of Th1 cells in initiating the immune response to fVIII, and of Th2 cells in the development of strong inhibitor production (16). Another study that conducted by Xuebin Ji in 2014 and they showed that this disease is an immune mediated disease of adults and children that is characterized by excessive platelet destruction and decreased platelet production by T1 cell (Th1 and Tc1) function (13). The aim of current study was firstly to investigate the role of cell mediated immunity response by measuring the ratio of CD4/CD8 counts in children patients suffering from hemophilia who referred to the Ali Asghar Hospital of Zahedan city, Zahedan-Iran and secondly to investigate the ratio of CD4/CD8 counts in association with some variables factors such as sex, age, type of hemophilia, disease intensity and type of treatment.

The Method Of Study:

This study was carried out on 80 children suffering from hemophilia disease referred to the Ali EbneAbitaleb and Ali Asghar hospitals of Zahedan city, Zahedan-Iran. 80 healthy individual sex and age matched were included. After obtaining the basic personal information, informed verbal consent was obtained from each family of subjects. The exclusion criteria was included as history of viral infections, gastrointestinal infections, and having any chronic infection (autoimmune diseases). These patients were investigated based on some variables such as type of hemophilia, gender, age, disease intensity. Two milliliters of EDTA ant coagulated whole blood was collected. Immunophenotyping of lymphocytes count was carried out by FACS (Fluorescent Antibody Cell Sorter, Becton Dickinson, Singapore, BD) using a double CD4 and CD8 kit (Model: FR868). Based on the instruction of kit, 100 µl from each samples were taken and added to clot tube and added 10 µl of antibody. Again 100 µl from the same sample were added to the clot tube separately and added 10 µl of control sample. Then these two samples were incubated for 30 minutes. Then 100 µl of Reagent A (UNIQUE-LYSE Code A100) were added and vortexed well and incubated at room temperature for 10 minutes in dark room. After one ml reagent B were added to the tube and incubated for 20 minutes in dark room. After these stage, the cytometry and cell counts were carried out within two minutes and the results were read later on. The sample cells supernatant were analyzed by flow cytometry instrument (PARTEC PAS II). All samples were stained on the same day and tested within two hours. 488 nm blue laser that read the color range between 520–570 and 670. The values of mean and standard deviation of each lymphocyte subpopulation were estimated. The mean ±

SD of absolute numbers of CD4 and CD8 lymphocytes/ml were obtained. Based on T-independent test, the mean CD4/CD8 ratio in patients with different variables were calculated and test of significance was done by calculating the standard error of difference between two means. The significant correlation between CD4/CD8 ratio and some variables were evaluated by statistical test and SPSS software and P value of less than 0.05 was considered significant.

Results:

From 80 hemophilia patients, we found 100% were type A hemophilia. Among of them 66 (82.5%) were male. Their age was between 1 and 45 years old with mean and standard deviation of 15 ± 3.51 . 12 (15%) of them had mild disease intensity and 68 (85%) had sever disease intensity. The number CD4 count was between 100 and 1610 with the mean and standard deviation of 568.22 ± 289.18 and the number of CD8 count was between 115 and 1430 with the mean and standard deviation of 349.61 ± 217.16 . The ratio between these cells was varied between 0.54 to 4.44 with the mean and standard deviation 1.79 ± 0.78 (Table 1).

Table 1
description of some variables among patients

Variables	Related variables	Number	Percent
Gender	Male	66	82.5
	Female	14	17.5
Disease intensity	Mild	12	15
	Sever	68	85

Based on T-independent test, the mean CD4/CD8 ratio in patients with severe were higher than mild disease intensity (P Value = 0.019). There was also not significant differences between the CD4/CD8 ratio with the gender (P Value = 0.095).

Table 2
The comparison of CD4/CD8 ratio based on different variables

Variables	Related variables	Mean \pm SD	P Value
Gender	Male	1.703 ± 0.699	0.095
	Female	2.118 ± 1.098	
Disease intensity	Mild	2.289 ± 1.093	0.019
	Sever	1.712 ± 0.698	

Discussion:

Hemophilia disease is a genetic bleeding disorder that causes recurrent bleeding in to the all part of body mainly in to the joints, muscles, brain. The disease is related to the function of immune system and the role of cell mediated immunity (CMI) especially the role of CD4 and CD8 lymphocytes in this disease are not well understood therefore we design this study to evaluate the T helper (Th) to T cytotoxic (Tc) ratio in children suffered from hemophilia disease referred to Ali Asghar hospital of Zahedan city, Zahedan-Iran. The results of this study were not found in any literature therefore this study provides the first estimates of CD4 and CD8 T lymphocyte counts and CD4/CD8 ratio among the hemophilia patients in southeast of the country. Regarding to the role of the ratio of CD4, CD8 in hemophilic patients, there was no study reported yet. The only study on the role of T cell immune abnormalities in immune thrombocytopenia as an autoimmune disease has been reported by Xuebin Ji et al in 2014. They showed immune thrombocytopenia is a disease of with abnormal T cell immunity, cytotoxic T cells, abnormal T regulatory cells, helper T cell imbalance, megakaryocyte maturation abnormalities and abnormal T cell anergy are involved in the pathogenesis of this condition (13). A wide variations in mean CD4 and CD8 count have been reported from studies conducted by Krishna Ray, S.M. Gupta et al in normal healthy individuals, HIV positive individuals and AIDS patients (17). In clinical status, the ratio of CD4 and CD8 ratio has been reported by Wei Lu and et al in 2011 in virologically suppressed HIV-positive patients (18). Their results indicated that the CD4:CD8 ratio can contribute to the immunological evaluation and this may has a role for monitoring both immune dysfunction and viral reservoir size in immune-based clinical trials. There is also another study concerning the CD4:CD8 ratio in association with markers of age associated disease in virally suppressed HIV-infected patients with immunological recovery (19). It is also reported the association between the CD4/CD8 ratio and carotid intima-media thickness (cIMT) progression in treated HIV-infected patients as a marker of coronary heart disease. The result indicated that can be clinically useful as predictor of cardiovascular events (20). So far, there are no large-scale studies with sufficient statistical power to clearly assess the association between the CD4:CD8 ratio with other pathological conditions. None of these studies did not examine the role of CD4 and CD8 individually and in combination with the ratio of these cells. On the other hand as the CD4:CD8 ratio is considered a marker for both immune senescence and immune activation in pathogenicity of disease, so we conducted a study focused on factors contributing to CD4:CD8 T cell ratio and clinical outcome in children hemophilia patients in the context of some epidemiological. Usually the CD4:CD8 ratio of less than 1.0 is considered as a surrogate marker of immunosenescence and represents an independent predictor for disease or healthy statuses. Hemophilia disease like other clot abnormalities such as ITP could be a T1 cell (Th and Tc) predominant disease although the precise mechanisms await further functional assay. Our results indicated that the ratio of CD4 and CD8 was obtained between 0.45 and 1.44 with mean 1.79 ± 0.78 and correlation with the gender and disease intensity was 0.095 and 0.019 respectively. Similar to these finding can be seen in review report of (19). They reported multivariate analyses adjusted in a case control study performed for age, sex, nadir CD4, proximal CD4 T cell count, year of ART initiation and ART duration on 407 patients for the prediction of non-AIDS events, including malignancies, cardiovascular and kidney diseases. Their results showed low CD4:CD8 ratio that was an independent factor for both non-AIDS morbidity and mortality in long-term treated HIV-positive patients and was independent of nadir CD4 T cell count. Finally, our study indicated that CD4 and CD8 T cell count and their ratio can be

predictive value for disease and may provide a surrogate marker of these cells cytokine balance and shifting the cytokine patterns from each of them and this might provide a potential immunotherapy program for hemophilia disease.

A larger group should be studied to thoroughly assess the variations in lymphocyte subpopulations in this patients.

Conclusion:

The results showed that CD4/CD8 ratio has correlation with disease intensity whereas CD4/CD8 ratio has no correlation with gender.

Abbreviations

T helper (Th)

T cytotoxic (Tc)

hereditary coagulation bleeding (HBD)

FXIII deficiency (FXIIID)

factor X (FX)

factor IX (FIX)

human coagulation factor 9 (hFIX)

factor FVIII (FVIII)

cell mediated immunity (CMI)

cytotoxic T cells (CTL)

T regulatory cells (Tregs)

helper T (Th)

carotid intima-media thickness (cIMT)

Declarations

Ethics Approval and Consent to Participate: not applicable.

Consent for publication: not applicable

Availability of data and supporting materials section: Please contact author for data requests

Competing Interests: Neither of the authors has any conflict of interest to disclose

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Author contribution:

Hk, MN,GM and AA participated in Conception and design of the study, library searches and assembling relevant literature, critical review of the paper, supervising writing of the paper, database management. The remained authors participated in data collection, library searches and assembling relevant literature, writing the paper, and critical review of the paper. All the authors have read the final version of the manuscript and approved it.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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