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Role of T regulatory Cells with Valvular Heart Disease.

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ABSTRACT

The present study was conducted to investigate the associated of t-reg. cells with valvular heart disease, which occurs as a result of different heart disease including (endocarditis, pericarditis and myocarditis) in addition to other diseases, eighty blood samples of patients suffering from various heart disease were collected from hospitals of middle Euphrates region at the period from November 2014-to- May 2015. Flow cytometry technique was used to measure level of T regulatory cells in the blood of patients and determine the proportion of T cells and their markers (CD4, CD25 and Foxp3). Patients show significant difference in CD4, CD25 and Foxp3 rate than controls, while no significant difference in T helper cells level in the same study group. The highest percentage of cells T reg recorded in Coronary Artery Disease(CAD) , Congenital Heart Disease (CHD) and Pericarditis by particularly aged over 50 years (47.5%), followed by age groups ranging between (41-50 years)with rate 22.5%.

Keywords: Heart disease , Valves, T-regulatory ,Foxp3, CD25.

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INTRODUCTION

Cardiovascular disease were the most commonly to cause of death at the global level in 2008, and were the responsible for 30% of cases, from these deaths more than three quarters were occurred of stroke and coronary artery disease (Longo *et al* ,.2011; WHO,.2014).

Heart's valve disease influenced with several risk factors including smoking ,high cholesterol in blood, alcohol, hypertension, congenital heart disease, chronic disease and infections by germs (Dantas *et al* ,.2012), diet, raised blood glucose levels, depression, physical inactivity, family history, obesity, stress age and gender(Hoen *et al* ,.2013,WHO,.2014). In cardiovascular disease the innate immunity is important because it's cells express various kinds of receptors which release during pathological condition (Ionita *et al* , 2010) ,and adaptive immune response may has role in development of heart diseases (Campbell *et. al*, 2012). Lymphocytes T cells are maturing thymus gland and release in blood stream with unique antigen and two kinds of T cells which bear the co-receptors CD4 or CD8(Cohn *et al.* , 2014). T cell activation is dependent on signals from receptors which can divided into three types (cytokine) receptor , co-stimulatory receptor and T-cell antigen receptor (Alarcon., 2014) .

T helper 17 is another regulatory T cell subset, (CD4 and CD25) are major lymphocytes which engaged in the maintaining tolerance in several models of autoimmunity and control of self-reactive. foxp3 are intracellular molecules and it is a functional marker on T-reg cells and it play role in Regulatory T cells which are an acomponent of immune system ,T-reg come in many forms as CD4,CD25 and FOXP3and T-reg cells are prevents the development of autoimmune disease (Singh *et al*., 2013). recently CD4 , CD25 and foxp3 are the most common accepted phenotype for T-reg (Sakaguchi *et al* ,.2010). Flow cytometry is a technology which is used for analysis the chemical and physical properties of cells when it passed in fluid stream through beam of light and by flow cytometry can measured (cell count and shape and structure of cell and cell size and DNA content and cell cycle phase and the absence and existence of specific proteins in the cytoplasm or on the cell surface and also flow cytometry used in medical diagnosis associated with disease like AIDS and cancer and other diseases (Zankl., 2012) .

MATERIALS AND METHODS

This study were conducted in The Middle Euphrates region Hospitals at the periods from October 2014 –May 2016. Eighty patients suffering from different types of heart diseases according to physicians diagnosis were included in this work, blood samples of them were obtained by venipuncture. For mononuclear cells isolation, part of these blood were added to heparin in a plane tube then lymphoprep density gradients solution of density 1.077 g/ml in a 50 ml sterile Falcon tube were used. the mononuclear cell layer that separated by former method were removed to a fresh tube, washed with RPMI 1640 then lymphocytes viability were determine using tryban blue stain (Blasiak ,2002). Flow-cytometry analysis and T-reg. phenotyping assay were done in Abu-Ali clinical center , Mashhad –Iran by using Bioscience Sandiago CA,USA.

Statistical analysis for T-cells and CD markers distribution were done using analysis of variance and t-test according to Statistical Package for the Social Sciences (SPSS) version 16(SPSS Inc. IL. USA). The results were expressed as Mean± SD .p-value below 0.05 were considered to be statistically significant (Chandel,2002).

RESULTS

Flow cytometry assay reveals that the mean of Tregulatory (CD4,CD25 and FOXP3) was (1.46), and the mean of T-helper (CD4 and CD25) was (2.44±1.40). table (1).

Table(1) Frequcy of Tcells by Flow

Variable	N	Mean± SD
Tregular (CD4,CD25 and FOXP3)	17	1.46±0.56
T Helper (CD4 and CD25)	17	2.44±1.40

The mean of T regular(CD4, CD25 and FOXP3) for patients was (1.46) while in control was (1.13) then it was significant of T-reg. by patients and control with P value 0.031 , while the mean of T Helper (CD4 and CD25) and Tcell (CD4 and FOXP3) for patients were(2.44) (2.26) while in control was (2.02) (2.16) respectively ,thus there were no significant with P value 0.232 of T helper by Patients and Control, p value ≤ 0.05 are significant. (table 2, figure 1)

Table 2 Mean Differences of T reg and T Helper by Patients and control

Flow-cytometry	group	N	Mean	S.D	t-test	p value
T regular (CD4, CD25 and FOXP3)	Patients	17	1.46	0.56	2.255	0.031
	Control	17	1.13	0.23		
T Helper (CD4 and CD25)	Patients	17	2.44	1.40	1.219	0.232

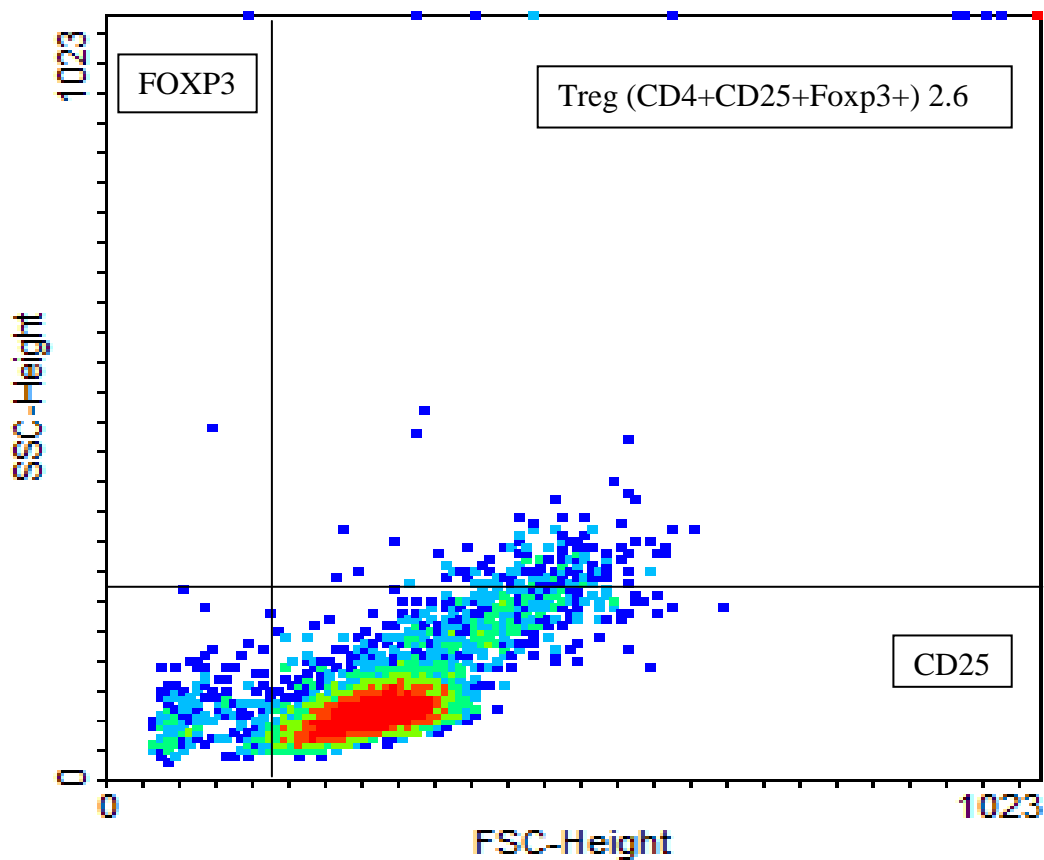


Figure (1) The frequency of CD4,CD25 and FOXP3 in patient

The association of disease with T regular and T Helper :

The T regulatory cells has the highest percentage (14.55%) more than T helper (3.43%) and T-cell (0.45%) ,thus the association of disease such as Coronary Artery Disease (CAD) which appear with a percentage (23.5%) ,Congenital Heart Disease(CHD) (41.2%) and Pericarditis (5.9%) with T regular (CD4, CD25 and FOXP3) was significant with P value <0.001 , while the association of disease types with T Helper (CD4 and CD25) show no significant differences with P value <0.41 . (table 3, figure 2). p value ≤ 0.05 are significant.

Table (3) The association of disease with T reg and T helper

Variable	disease						χ^2	P values
	CAD (%)	CHD (%)	Endocarditis (%)	pericarditis (%)	TOF	myocarditis (%)		
T regular normal high	0 (0.0) 4 (100.0)	0 (0.0) 7 (100.0)	5 (100.0) 0 (0.0)	0 (0.0) 1 (100.0)	0(0.0) 0 (0.0)	0 (0.0) 0 (0.0)	14.55	0.001
T Helper normal high	3 (75.0) 1 (25.0)	2 (28.6) 5 (71.4)	3 (60.0) 2 (40.0)	1 (100.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)		

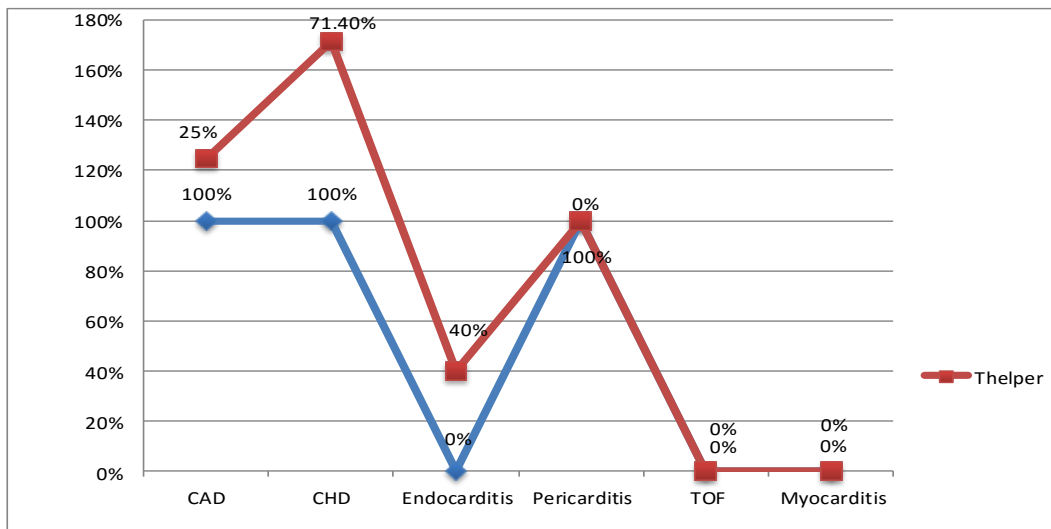


Figure (2) The association of disease with T reg and T helper.

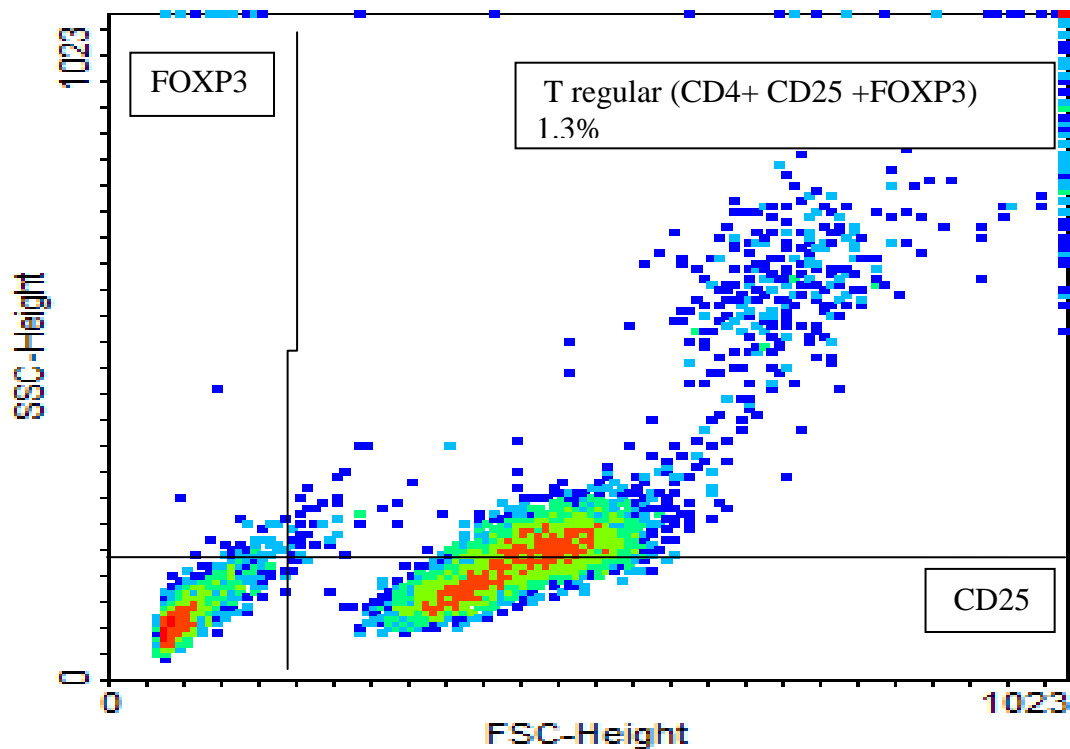


Figure (3) Flow cytometric characterisation of CD4^{pos}CD25^{pos} T cells in a representative patients (P).

DISCUSSION

Regulatory T cells play an essential role in immune reactions and determination of immunological relationships with several diseases particularly autoimmune disorders (Singh et al ,2013) . Results of the current study which existing in table (1) referred that a higher percentage of Treg appear in patients (1.46 ± 0.56) than control group (1.13 ± 0.23) with a significant differences (P value < 0.031). This may due to the role of T-regulatory cells in maintaining the balance and inducing the immune system. Treg cells is a major category of CD4+ T cells, which express the transcription factor Foxp3, and play a critical role in maintaining immune system homeostasis by suppressing the activation and expansion of self-reactive lymphocytes and therefore inducing autoimmune diseases (Hori et al ,2003). Foxp3 appears to function as a transcription factor and master regulator in development and function of regulatory T cells. (Fontenot et al ,2003). This results show disagree with other studies of relationship between Treg with rheumatic mitral stenosis , regulatory T cells count was significant lower in mitral stenosis patients than in control , and the researchers was suggested a decrease in the numbers of T reg cells in patients might suggest a role for cellular autoimmunity in a rheumatic process.(Yildiz et al ,.2007). The results show no significance in the percentage of T helper between the patients (2.44 ± 1.40) and control (2.02 ± 0.24) with P value < 0.232 .due T helper not associated with VHD ,which agree with finding of(Soejima et al.,2003) , this results disagree with other study in Turkey which pointed that T helper increase with mitral valve disease patients comparing with control .T helper not associated with VHD ,which agree with finding of(Soejima et al.,2003) , this results disagree with other study in Turkey which pointed the T helper increase with mitral valve disease patients comparing with control (Bas et al ,.2014). In present study ,results T reg cells which existing in table (4-18) show high of T reg (14.55%) more than T helper and Tcell (3.43%)(0.45%)respectively ,therefore the association of disease types with T reg was differs according to the disease types such as(Coronary Artery Disease CAD and Pericarditis . T regular (CD4, CD25 and Foxp3) show significant differs with this diseases, P value < 0.001 . This results agree with another studies about Treg with Coronary Artery Disease which pointed a significant increase in activated lymphocytes in patients in comparison to control group , and significant decrease in T-reg in patients comparing with control (Romuk et al ,.2014). Whereas ,the current study disagree with another study which pointed a chronic inflammation atherosclerosis that refer to decrease in the numbers of Treg (Zhang et al.,2012) . Contradictory results have been recorded about increase or decrease in Treg numbers in patientswit CAD may be due to the experimental method and quality of flow cytometry based identification of Treg cells (Jevallee et al.,2011), or the increase in numbers of T reg might be to aimed at limiting the cardiovascular damage or alternatively a proinflammatory state preceding the acute coronary disease, all this still unclear and requires more investigation (Ammirati et al.,2010). Also the relationship between Endocrditis and Treg cells was increased in patients with Q fever that mean is significant compare with control , the researchers were suggested that the expansion of T reg may be critical of the chronic evolution of Q fever (Layez et al ,.2012). Finally , despite the exact molecular mechanisms underlying the cardioprotective effects of Treg cells are yet to be elucidate and need to more studying , targeted treatments with Treg cells might supply a promising and novel future approach to the prevention and therapy of cardiovascular diseases(Meng et al.,2015). T Helper (CD4 and CD25) show no significant with P value < 0.41 .due the change in frequency of the T helper no associated with the severity of the narrowness of the coronary artery, but it associated with the the inflammatory status and plaque of coronary artery disease. This results agree with finding of (Lin et al.,2013) .

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