Original Article

Anti-inflammatory Role of IL-4 in Patients with Rheumatic Mitral Valve Stenosis

Nidhal Abdul-Muhaimen* **BSc, MSc, PhD**
Zaman I. L. Al-Kaabi** **BSc, MSc**

Summary:

Background: IL-4 is an antibodies inflammatory cytokine which has an important role in protecting against the inflammatory reactions in most of diseases. Here, we try to highlights the role of this cytokine in chronic Rheumatic heart disease and its correlation with the extent of histopathological abnormalities.

Patients and Methods: Rheumatic mitral valve surgical fragments were taken from a total of 48 Iraqi patients with chronic rheumatic heart disease under mitral valve replacement surgery in Ibn Al-Bitar Hospital for Cardiac Surgery-Iraq-Baghdad. Paraffin embedded mitral valve tissue sections were prepared. IL-4-expressing cells were detected by using immunohistochemical staining technique and histopathological picture was studied by using hematoxylin and eosin staining.

Results: There were lower numbers of IL-4 positive cells in all patients under study in general, but very lower IL-4 positive cells percentage was recorded in high risk group patients. There was no significant difference in the distribution of IL-4 mean positive cell's count among all patients ($p > 0.05$). There was a correlation of IL-4 with the extent of histopathological abnormalities, and odds ratio results displayed that the histopathological abnormalities which recorded in the case group 4 times than that occurred in the control group.

Conclusion: IL-4 plays an important role in regulating the inflammatory responses against the heart in chronic rheumatic heart disease.

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Introduction:

Rheumatic heart disease (RHD) is the most common cardiovascular disease affecting children and young adults in the world,1 and till now it is consider a major public health problem causes about 400000 deaths annually worldwide especially in developing countries.2 Molecular mimicry between heart tissue proteins and streptococcal antigens, mainly the M protein which is the major component of the streptococcal cell surface and the most important virulence factor, has been proposed as the triggering factor leading to autoimmunity in rheumatic heart disease patients.3 In rheumatic heart disease patients several studies suggest that there is a scarce production of regulatory cytokines, such as IL-4, in the valvular tissue. Eighty-two percent of valvular fragments (mitral and/or aortic valve) showed a low frequency (<10%) of IL-4-producing cells, whereas 78% of myocardial tissue fragments showed cells capable of producing IL-4.4 Here, we try to study the correlation between IL-4 (as an anti-inflammatory and regulatory cytokine) and the extent of histopathological abnormalities in chronic rheumatic heart disease Iraqi patients.

Patients and methods:

This study was conducted from October 2006 to September 2007. Rheumatic mitral valve surgical fragments and blood samples were taken from 48 patients with chronic rheumatic heart disease under mitral valve replacement surgery in Ibn Al-Bitar hospital for cardiac surgery\Baghdad\Iraq. All patients were divided according to the positive or negative history of rheumatic fever (PHORF and NHORF), PHORF patients were subdivided according to the frequency of rheumatic fever, and according to the period of medication treatment into single attack under continuous medication (SA^{UCM}), single attack without continuous medication (SA^{WCM}), high risk under continuous medication (HR^{UCM}), and high risk without continuous medication (HR^{WCM}). Controls for mitral valve tissue samples were taken as a negative controls from 20 cadavers, their cause of death not related with acute or chronic rheumatic heart disease, infective endocarditis or any other heart disease, and their age and sex were matched with chronic rheumatic heart disease patients. Paraffin embedded mitral valve tissue sections with 5µm thickness were prepared using positive charge slides (Fisher Scientific, USA) Hematoxylin and eosin (H&E) staining was performed on mitral tissue sections for each patient. Rat anti-human IL-4 protein (Serotic, UK) was used to detect IL-4 expression in mitral valve infiltrating mononuclear cells by using immunohistochemical staining technique.

Statistical Analysis*:* IL-4 signals were evaluated by counting the number of positive cells which were adheres to the valvular endothelium and the total

**Dept of Microbiology, College of Medicine, Al-Nahrain University.*
*** Dept. of Microbiology\Immunology, College of Dentistry, Babylon University.*

number of infiltrating cells in 5 to 50 microscopic fields to measuring the percentage of positive cells as follows: The percentage of positive cells = The number of positive cells / The number of total cells X 100. The immunohistochemistry signals of IL-4 expression on infiltrating cells demonstrating on the mitral valve tissue sections were considered +, <10%; ++, 10 to 50%; and +++, >50% as positive cells.[4] All statistical analysis was performed with the SPSS 10.01 statistical package for social sciences and also Excell 2003. A p value of less than 0.05 ($p < 0.05$) was considered the level of significant.

Results:

IL-4 immunohistochemical staining was carried out on all specimens under study, and the results were arranged in (Table 1) showed that there was a low predominance for IL-4 positive cells in the mitral valve tissue in all chronic rheumatic heart disease patients in general. The mean percentage of IL-4 positive cells in the SA^{UCM} group (15.9%) was higher than that of SA^{WCM} patients (7.83%), but we found that the expression of IL-4 in the HR^{UCM} group (3.6%) was lower than HR^{WCM} group (6.72%). NH patients showed high mean percentage (14.9%) when compared with SA^{WCM}, HR^{UCM}, and HR^{WCM} groups, and it was appeared near to the mean percentage of SA^{UCM} group. However, the comparison between study population group by using Chi-square test revealed that there was no significant difference in the distribution of IL-4 mean positive cell's count ($p > 0.05$).

Table (1): Mean percentage of IL-4 positive cells from the total number of mitral infiltrating mononuclear cells among different study groups.

Group type					IL-4 +ve cells				
					No.	Mean % ±SD*			
		14	20.59	32.86	4.29	14.9±12.579	0	36.36	++
	SA^{UCM}	5	7.35	28.4	4.2	15.9±7.632	9.67	28.58	++
	SA^{WCM}	18	26.47	36.44	2.22	7.83±8.995	0	28.57	+
	HR^{UCM}	4	5.88	26.26	1	3.6±3.305	0	8	+
	HR^{WCM}	7	10.29	51.43	3.14	6.72±7.681	0	18.75	+

$\chi^2 = 3.099$, $P > 0.05$; ICsC* = Infiltrating Cells Count; SD* = Standard Deviation; Min.* = Minimum; Max.* = Maximum; Total value* = + (< 10) %, ++ (10-50) %, +++ (≥ 50) %.

There were (43.75%) from the total number of chronic rheumatic heart disease patients distributed among NH, SA^{UCM}, SA^{WCM}, and HR^{WCM} groups were displayed (> 10 < 50%) of IL-4 positive cells, whereas, there were (25%) of patients had less than (10%) of infiltrating mononuclear cells were positive for IL-4 cytokine production. None of chronic rheumatic heart disease cases were displayed (50%) or more than (50%) of IL-4 positive cells.

Table (2): Frequency of expression, relative risk (RR), and odds ratio (OR) of IL-4 in case/control groups.

			Positive	Negative	Positive	Negative	
Case	NHORF	No. (%)	10(71.43%)	4(28.57%)	25(64.1%)	14(35.9%)	39(100%)
	HR^{WCM}	No. (%)	4(57.14%)	3(42.86%)			
Control					8 (88.9%)	1 (11.1%)	9 (100%)
	HR^{UCM}	No. (%)	3(75.00%)	1(25.00%)			
OR (Odds Ratio)					0.2	4.5	

Negative IL-4 expression was found in (31.25%) of patients among all study population group. To study the correlation between the extent of histopathological abnormalities with the presence of IL-4 which was known as a regulatory anti-inflammatory cytokine, we calculate the odds ratio (OR), which represent the relative risk (RR) for the presence versus absence of IL-4, thus, we considered the patients without continuous medication (NHORF, SA^{WCM}, and HR^{WCM}) as cases, whereas patients under continuous medication (SA^{UCM} and HR^{UCM}) were considered as controls (Table 2). The results above were displayed that the histopathological abnormalities which recorded in the case group (NH, SA^{WCM}, and HR^{WCM}) is 4 times than that occurred in the control group (C-group) (SA^{UCM}, and HR^{UCM}). Immunohistochemical staining for human IL-4 was illustrated in (Figure 1).

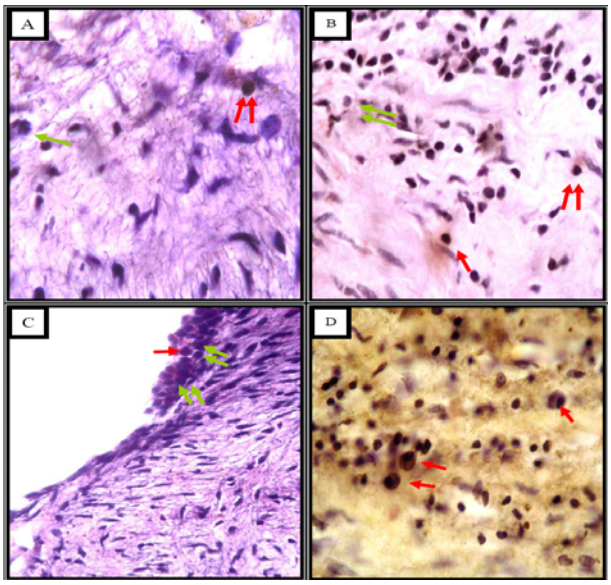

Figure (1): Immunohistochemical staining of chronic rheumatic mitral valve tissue sections for human IL-4 cytokine secrete by infiltrating mononuclear cells. (A&D) showing IL-4 positive cells (< 10%) (+), (B&C), IL-4 positive cells comprised (10-50%) (++). Red arrows indicate the positive cells, whereas IL-4 negative cells are indicated by green arrows. Microscopic magnification power: X1000 (A&D); X400 (B&C).

Discussion:

IL-4 which was known as an important anti-inflammatory cytokine in determines the excessive autoimmune inflammatory reactions. However, our results showed that there were a small number of IL-4-producing cells in the tissue sections of mitral valve and no significant difference was found in the mean percentage of IL-4 positive cells among all groups under study, and this implicates a downregulation of inflammatory responses, leading to the progression and maintenance of mitral valve lesions. The lower production of IL-4 from mitral-valve infiltrating mononuclear cells was found to correlate with the severity of histopathological abnormalities, and this may be as a results of two points, the first one is that IL-4 has the ability to reverse the inflammatory reaction and the low level of this cytokine lead to increase the inflammatory response against the heart, and the second one is that IL-4 is very important for the development and function of CD4+CD25+ nTreg cells. The modulatory effects of interleukin 4 (IL-4) on the function of Tregs has been explored in many studies. IL-4 was shown to prevent the spontaneous apoptosis and the decline of Foxp3 mRNA which were found to occur during culture of isolated nTregs.5 It has been reported that IL-4 prevents death of resting T cells.6 nTregs are known to rapidly die in vitro,7 and to test whether IL-4 could also preserve viability of nTregs, CD4+CD25+ T cells were incubated in medium with or without IL-4, the percentage of living cells was significantly higher for Tregs incubated with IL-4 compared to nTregs incubated in medium alone. nTregs exposed to IL-4 were more potent in suppression the proliferation of naïve CD4+ T cells and they better inhibited IFN-γ production by CD4+ T cells as compared to nTregs cultured in medium. IL-4 also enhanced membrane IL-2Rα (CD25) expression on nTregs above the levels observed on freshly isolated cells. IL-4-mediated effects on nTreg function persisted in Tregs from Stat6 deficient mice, pointing to a Stat6-independent intracellular transduction pathway. Many studies suggest that the anti-inflammatory function of IL-4 could partly be mediated by effects on nTregs function. Therefore, any reduction in the production of this cytokine may affect the suppressive function of nTregs against CD4+ T cells leading to more damage to the heart especially the mitral valve, and this appeared visible in the significant correlation between positive/negative IL-4 cells and the extent of histopathological abnormalities (OR = 4.5).

Conclusion:

IL-4 play an important role in reversing the inflammatory state and regulating the autoimmunity in rheumatic heart disease because our results showed that the presence of IL-4 correlate with the severity of histopathological abnormalities.

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