TNF-α and Autoimmunity in Chronic Rheumatic Heart Disease

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Summary:

Background : We Know that TNF-α Which is Consider an important inflammatory Cytokine mediate the inflammatory reactions which occur in the tissues against the infections agents and other causes of inflammation therefore , here , We try to study the role of TNF- α in Chronic Rheumatic heart disease and the relationship of TNF-α with extent of histopathological abnormalities .

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. TNF-α -expressing cells were detected by using In Situ Hybridization technique, and histopathological picture was studied by using hematoxylin and eosin staining.

Results: High percentage of TNF-α positive cell was found in all patients in general. There was a significant difference in the mean percentage of TNF-α among all groups under study ($p < 0.05$), and there was a positive high significant correlation was found between the number of TNF-α positive cells and the extent of histopathological abnormalities.

Conclusion: TNF-α play an important role in heart damage development due to its significant correlation with the extent of histopathological abnormalities.

Keywords: TNF-α, In-Situ- hybridization, Chronic Rheumatic Heart Disease

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Introduction:

Cachexin or cachectin and formally known as tumor necrosis factor-alpha is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. The primary role of TNF is in the regulation of immune cells. TNF is also able to induce apoptotic cell death, to induce inflammation, and to inhibit tumorigenesis and viral replication. Dysregulation and, in particular, overproduction of TNF have been implicated in a variety of human diseases, as well as cancer.(1) In rheumatic heart disease, the evaluation of proinflammatory cytokines levels produced by peripheral blood mononuclear cells from rheumatic fever\rheumatic heart disease patients without congestive heart failure after streptococcal antigen and pokeweed mitogen stimulation showed increased production of TNF-α, IL-1 and IL-2. Low levels of these cytokines were produced by tonsillar mononuclear cells from the same patients.(2) Interestingly, the production of TNF-α was correlated with the progression of Aschoff nodules in the valve lesions of acute rheumatic fever patients.(3) Here, in this study, we try to detect the role of TNF-α in enhancing heart damage in chronic rheumatic heart disease Iraqi patients.

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. 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. Biotinylated long DNA probe for human TNF-α (Maxim Biotec, Inc., USA) was used to detect TNF-α expression in mitral valve infiltrating mononuclear cells by using In Situ Hybridization technique (ISH).

Statistical Analysis: TNF-α signals were evaluated by counting the number of positive cells which were adheres to the valvular endothelium and the total 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. In Situ Hybridization signals of TNF-α expression on infiltrating cells demonstrating on the mitral valve tissue sections were considered +, <10%; ++, 10 to 50%; and +++, >50% as

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positive cells.(4) All statistical analysis was performed with the SPSS 10.01 statistical package for social sciences and also Excel 2003. A p value of less than 0.05 ($p < 0.05$) was considered the level of significant. (4)

Results:

Hematoxylinandeosinstainedtissuesectionsofrheumaticmitral stenosis appeared different degrees of fibrosis, inflammatory cellular infiltrates, neovascularization and mineralization. More details about chronic rheumatic heart disease patients and their numbers, clinical and histopathological picture are shown in (Table1), (Figure1), and (Figure 2).

Table (1): Clinical and histopathological characteristics among different study population groups.

Group type			Patients					
			No.	(%)		F^a	C^b	CF^c
NHORF			14	20.59	MD ±AR	++ ++	++	++ ++
PHORF	SA	SA^{UCM}	5	7.35	MS or MR	++ +	++	+/-
		SA^{WCM}	18	26.47	MD ±AR	++ ++	++ +	++ +
	HR	HR^{UCM}	4	5.88	MS or MD	++ +	++ +	++
		HR^{WCM}	7	10.29	MAD ±TR	++ ++ +	++ ++ +	++ ++ +
Control			20	29.41	N	-	-	-

F^a = Fibrosis, C^b = Calcification, CF^c = Cellular Infiltration; M = Mitral; A = Aortic; T = Tricuspid; S = Stenosis; R = Regurgitation; D = Stenosis and regur- gitation; N = Normal; + = 10%, ++ = 20%, +++ = 30%, ++++ = 40%, +++++ = 50%.

The clinical features were graduated in severity among all patients due to the continuous inflammatory processes against the heart; as a result, highly significant differences were shown in histopathological features among different study groups when compared between them. In general high risk patients were display very large degree of histopathological abnormalities (fibrosis, calcification, and cellular infiltration) which reflects the more severity of disease than other groups, and there was a highly significant difference between the degree of cellular infiltration in the patients of HR and SA- groups ($p < 0.01$). Medical care groups (SA^{UCM} and HR^{UCM}) were exhibited no or very low-grade of the inflammatory response and subsequently low heart lesion, fibrosis and calcification when compared with patients of intermittent medical therapy (SA^{WCM} and HR^{WCM}).

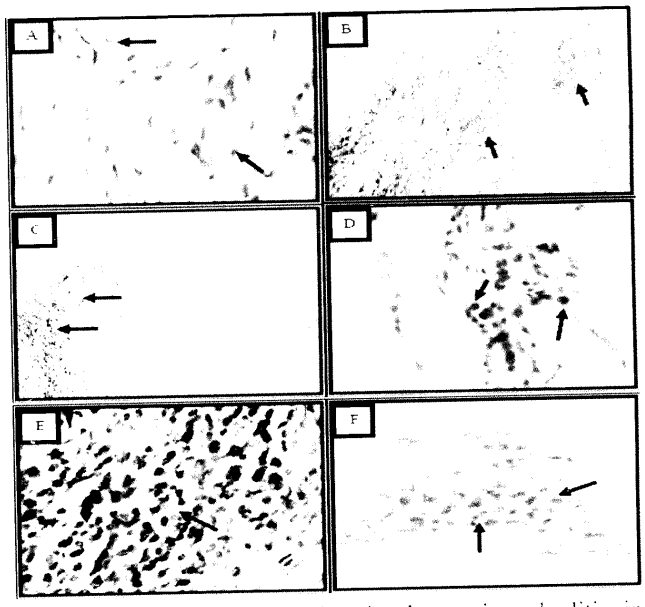

Figure (1): Illustration of chronic rheumatic valvulitis in (H&E)-stained mitral valve tissue sections. (A&B), showing autoimmune lesions in the mitral valve of patient No. 31, cellular infiltrations are indicated by long arrows and short arrows indicate Aschoff bodies. (C, D, E, and F), showing disruption of the myocardium with infiltrating mononuclear cells in patients number 27, 33, 38, and 41 respectively, vegetations are indicated by short arrows and mature cells infiltration by long arrows. Microscopic magnification power: (B and C), X100; (A, D, E, and F), X1000.

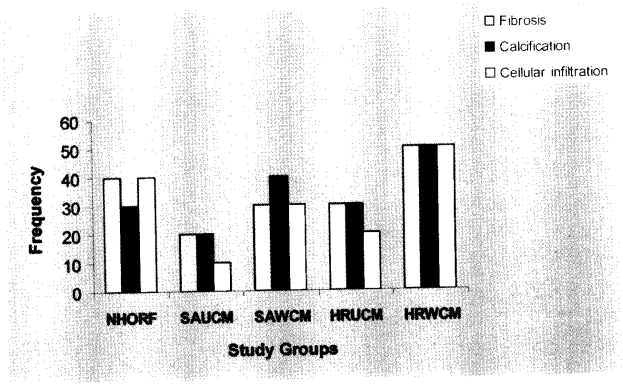

Figure (2): The degree of fibrosis, calcification, and cellular infiltration among different study groups.

There was no significant difference in the severity of disease between negative history and SA^{WCM} groups except the increasing in the cellular infiltration in the negative history population with subclinical symptoms which leads to more heart damage. Detection of TNF-α expression was carried out by using ISH technique, and according to the scoring system, the results shown in (Table 2) were displayed that more than 50% of mononuclear cells which infiltrates the mitral valve were positive for TNF-α in most of chronic rheumatic heart disease patients in this study (Figure 3).

Table (2): Mean percentage of TNF-α positive cell from the total number of mitral infiltrating mononuclear cells among different study groups.

Group type		Patients		ICsC*	TNF-α +ve cells		Min. (%)	Max. (%)	Total value*
		No.	(%)		No.	Mean% ±SD*			
NHORF		14	20.59	32.07	17.93	54.39± 14.360	30.43	75.67	+++
PHORF	SAUCM	5	7.35	25.8	4.4	16.65± 10.081	4	31.03	++
	SAWCM	18	26.47	37.44	23.94	61.33 ±13.258	44	84.61	+++
	HRUCM	4	5.88	28.3	11	33.91 ±26.216	8.33	57.14	++
	HRWCM	7	10.29	51	41.14	80.33± 4.240	74.46	86.44	+++

ICsC* = Infiltrating Cells Count. Total value* = + (< 10%), ++ (> 10% < 50%), +++ (≥ 50%). Min. = Minimum, Max. = Maximum. SD* = Standard Deviation.

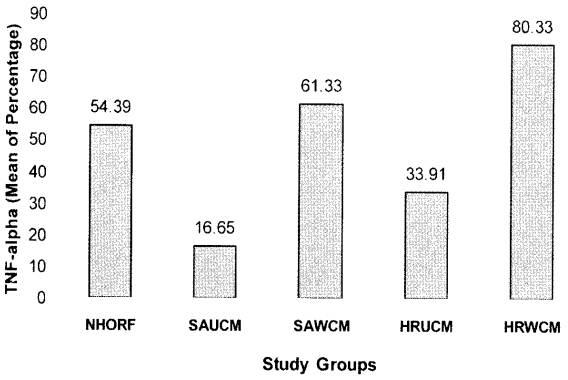

Figure (3): Mean percentage of TNF-α positive mitral valve infiltrating mononuclear cells among all study groups.

According to the results obtained from χ^2 analysis, there was a high significant difference in the mean percentage of TNF-α among all study groups ($\chi^2 = 10.39$) ($p < 0.05$) especially when we compared HR^{WCM} group (80.33%) with patients of single attack without continuous medication (61.33%), but both of them had higher numbers of TNF-α positive cells than HR^{UCM} (33.91%) and SA^{UCM} (16.65%) groups which also there was a difference between them when considered the numbers of acute rheumatic fever attacks in the first line. From the total number of chronic rheumatic heart disease patients (64.58%) had more than 50% of TNF-α positive cells, and about (35.42%) had less than 50% of infiltrating mononuclear cells were positive for TNF-α, among them there were only two exceptions who had less than 10% of TNF-α positive cells, the first one had (4%) (Patient number 3-SA^{UCM}), whereas the other had (8.33%) (Patient number 1-HR^{UCM}). None of patients of HR^{WCM} group have less than (50 or 10) % of positive cells and all of them had high predominance of TNF-α (more than 50%). NHORF group showed lower numbers of TNF-α positive cell (54.39%) than that in SA and HR groups without continuous medication. There was a large variation in the mean percentage between minimum and maximum value in negative history (30.43 and 75.67) % and this was also found in HR^{UCM} group (8.33 and 57.14) %. Although continuous medical therapy leads to prevent the recurrent attacks of acute rheumatic fever, some patients were displayed high degree of inflammatory response lead to more cellular infiltration and more TNF-α production. In HR^{UCM} group, two patients were exhibited more than 50% of infiltrating cells positive for TNF-α, one of them have 57.14% (patient number 2) and the other one was displayed 55.88% (patient number 3) of positive cells. TNF-α was known as a central mediator of inflammatory processes, therefore, the development of histopathological abnormalities in chronic rheumatic heart disease is correlated to a large degree with the amount of TNF-α. Therefore, positive high significant correlation was found between the number of TNF-α positive cells and the extent of histopathological abnormalities in all groups under study (Figure 4).

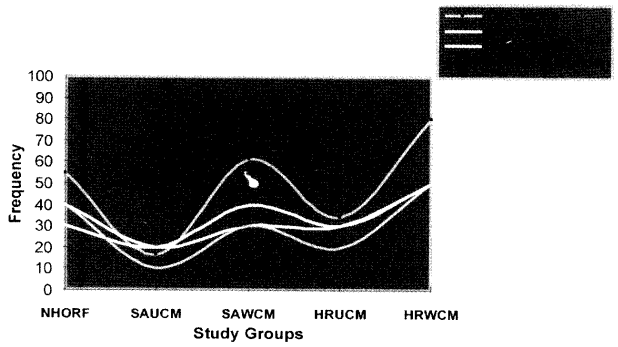

Figure (4): Correlation of TNF-α percentage with the extent of histopathological abnormalities among different study groups.

Anova test revealed that there was a significant difference between the mean percentage of TNF-α positive cells and the extent of histopathological abnormalities ($p < 0.05$) (Table 3), and highly significant difference was recorded between the degree of fibrosis and cellular infiltration ($p < 0.01$).

Table (3): The difference in the mean percentage of TNF-α in correlation with the extent of histopathological abnormalities.

Histopathological abnormalities		Fibrosis	Calcification	Cellular infiltration
TNF-α	rho	0.886	0.948	0.947
	P value	P<0.05*	P < 0.05*	P < 0.05*
Fibrosis	rho		0.808	0.971
	P value		P > 0.05	P < 0.01**
Calcification	rho			0.832
	P value			P > 0.05
Cellular infiltration	rho			
	P value			

*Significant, **highly significant (ANOVA test).

In Situ Hybridization test for TNF-α DNA probe was illustrated in (Figure 5) which display the highly prevalence of this cytokine in the mitral valve of chronic rheumatic heart disease patients. Positive cells appeared in bluish purple color when compared with the negative cells stained with the red color which accumulates in the nucleus of these cells.

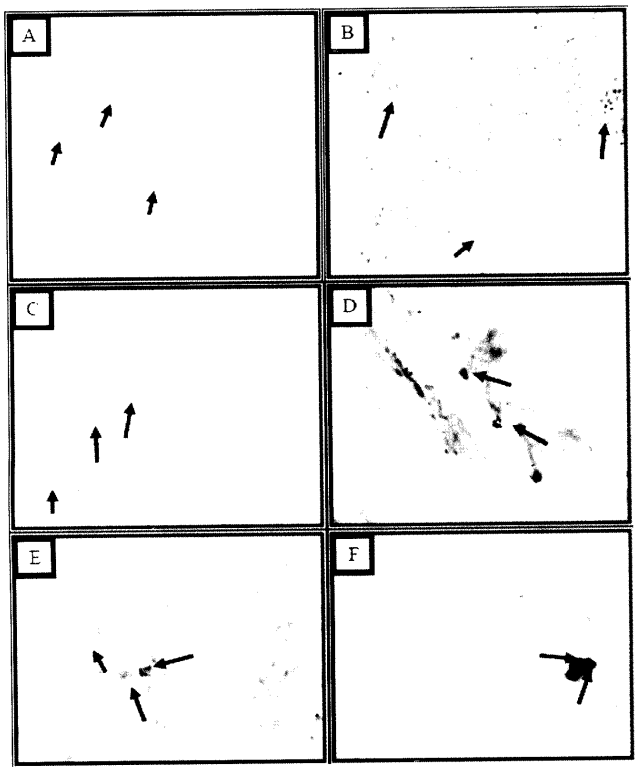

Figure (5): In Situ Hybridization in chronic rheumatic mitral valve tissue sections for human TNF-α cytokine secrete by infiltrating mononuclear cells. Tissue sections were stained by BCIP/NBT (bluish purple) and counterstained with nuclear fast red (NFR). (A) no using of TNF-α DNA probe (negative control), therefore, no signal is present, (B&E) showing TNF-α positive cells (< 50%) (+++), (C&F) TNF-α positive cells (10-50%) (++) from the total number of infiltrating mononuclear cells. Microscopic magnification power: X400 (B, and D); X1000 (A, C, E, and F).

Discussion:

In this study, TNF-α positive cells was detected in rheumatic mitral valve tissue sections by In Situ-Hybridization test and the results displayed the high percentage of infiltrating cells which produced TNF-α in most samples especially high risk patients, which revealed more damage due to recurrent attacks of acute rheumatic fever, whereas lower TNF-α positive cells was recorded in medical care groups and the high prevalence of TNF-α in some of patients from these medical care groups may return to increasing the inflammatory response due to new infection with group A β-hemolytic Streptococcus pyogenes and new subsequent acute rheumatic fever attack as a results of resistant to penicillin. While TNF-α has well-documented proinflammatory effects in endotoxic shock, tissue inflammation and autoimmunity, prolonged exposure to TNF- α can have anti-inflammatory and protective effects. Several studies suggest that CD4 + T cells were killed in a TNF-α-dependent manner in response to high-dose intravenous peptide, but only in the absence of intact Fas-FasL signaling pathways, suggesting that under certain circumstances TNF-α signaling provides a back-up death-effector pathway for T lymphocytes, (5) and this may explain one of many causes which we made ask; why high risk patients had more than one episode of acute rheumatic fever? In autoimmune disease, both short-term and chronic TNF-α exposure plays significant roles. Many studies suggest that both the targets and effector responses following TNF-α signaling depend on the duration of the response following binding of TNF-α trimers to trimerized TNF- αRs. Short-term exposure leads to acute inflammation where activation, chemotaxis and transmigration of cells predominate; host tissue may also be damaged. Acute inflammation may lead to resolution or to chronic inflammation. The latter situation leads to progressive impairment of effector functions and to a T cell phenotype which can be reproduced by chronic exposure to TNF-α resulting immunosuppression affects cognate immunity, abrogating the anti-self autoreactivity, but at the same time rendering the host susceptible to infection and impairing anti-tumor immunity. (6) This phenotype is a well-documented feature of many chronic inflammatory diseases. Acute and chronic responses will coexist in vivo, but the balance of these processes at any one time will be critical and will determine disease activity and progression. Also, these findings may lead to ask, what is the evidence that TNF-α is involved in cytocidal regulation either under physiological conditions or in autoimmune disease? Certainly, TNF-α is expressed in target tissues in vivo but when levels are examined in dissociated cell cultures they are at least an order of magnitude lower than the nanomolar concentrations that can kill T cells in vitro.(7) If TNF-α expression was central to regulatory programmed cell death pathways in Fas-/FasL-competent mice, one might expect to see expansions of T cell subsets in mice deficient in TNF-α or TNF-αRs$^{-/-}$ as originally reported in lpr (Fas-deficient) or g/d (FasL-deficient) mice.(8) These findings are perhaps less surprising in light of data demonstrating that TNF-α induced activation of the transcription factor NF-κB is antiapoptotic rather proapoptotic,(9,10) and suggest that TNF-α may suppress autoimmunity through alternative mechanisms other than activation of death pathways.

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