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ROLE OF PRO-INFLAMMATORY AND TOLL LIKE RECEPTOR-4 IN CARDIAC VASCULAR PATIENT IN NASIRIYAH CITY

Amany Sh.Jaber[1], Mayyada F. Darweesh[2]

[1]Department of pathological analysis, Collage of Science, University of Thi-Qar, Iraq.

[2] Department of Biology, Collage of Science, Kufa of University. Iraq

amanybiosciense@yahoo.com

Abstract

Cardiovascular disease (CVD) a major public health burden that lead to disability , morbidity and mortality. Inflammatory cytokines plays a crucial role in the pathophysiology of CVD. Therefore, this study aim to determined role of IL6, TNF-α and TLR4 on progression CVD in infected and non infected patients. A retrospective hospital study was performed to 280 patients with age range between 20-65 years attending to Heart Medical Center of Thi-qar province during 2018 divided into: 200 (71.4%) catheterized patients represent by diagnostic, therapeutic catheter and both .As well as 80(28.6%) surgery patients included Coronary artery bypass grafting CABG, valve replacement and underwent heart defects surgery. The Swabs, Catheter tip and blood samples were taken from all patients, surgery instruments and cultured on blood culture bottle for 24 h at 37ºC then sub-culture on enrichment and differential media. The results showed that positive bacterial culture appear in 62 cardiac catheterized patients and 18 of cardiac surgery patients, some specimens show poly-microbial growth. So the total isolates reach to 93 isolates, 75 of them were G +ve bacteria and 18 were G-ve bacteria and 200 specimens gave negative results for bacterial growth. The level of IL-6, TNF-a and TLR-4 estimated by ELISA and show a significant increase in CVD patients in compare with healthy control. The level of IL-6, TNF-a and TLR-4 highly increase in infected cardiac patients then in non-infected patients with statically significant different as well as in catheterized patients more than cardiac surgery patients with no significant difference between them. In conclusion : infection in CVD had a significant role in progression the disease that lead to highly increased in TLR-4 ,TNF- α , IL, 6 levels this reflecting the inflammatory response in the patients and causes progression the disease.

***Keyword*:** Cardiovascular diseases, Bacterial infection, IL-6, TNF-, TLR-4

Introduction

Cardiovascular diseases (CVD) a very complex set of diseases leading to outcomes related to acute or even emergency hospital care as well as to long-term monitoring of chronic diseases in lifelong follow-up (Collier and Kienzler, 2018). The World Health Organization (WHO) recorded about 20 million CVD deaths in 2015, accounting for 30 % of all deaths worldwide (WHO, 2015).

Infective endocarditis (IE) is an infection of the endothelial surface of the heart or intravascular and intra-cardiac devices like prosthetic valves and pacemaker cables (Thomas *et al.,* 2016). *Streptococci, Staphylococci, Enterococci*, and fastidious gram-negative *coccobacilli*, *Haemophilis* species a common pathogen associated with IE (Leonardo *et al*., 2006). Fibronectin binding proteins among *S. aureus*, and surface glucans in viridans streptococci have ability to adhere to the endothelial cells and stimulate the recruitment of phagocytic cells through induced surface expression of cell adhesion proteins and lead to an increased secretion of chemotactic cytokines and pro inflammatory cytokines such as TNF-α, IL-6 (Shaikh, 2011). IL-6, TNF-α and C-reactive protein (CRP) play important roles in the promotion of coronary artery disease (Ol KK *et al*., 2011), demonstrated that CVD patients are characterized by persistent immune activation *in vivo* which is reflected in increased circulating levels of inflammatory cytokines (TNF-α, IL-1β and IL-6) and chemokines (IL-8) within the failing myocardium, independent of the cause of Chronic Heart Failure.

TLR4 belongs to the family of pattern recognition receptors (PRRs). They are highly conserved receptors that recognize pathogen-associated molecular patterns (PAMPs), thus representing the first line of defense against infections, expressed on the cell surface on both hematopoietic and non-hematopoietic cells, including endothelial cells of cardiac myocytes (Kielian, 2006; Akira and Takeda, 2004). TLR4 plays a critical role in myocardial inflammation, including myocarditis, Myocardial Infarction (MI), myocardial I/R injury, Heart Failure(HF), aortic valve diseases, atherosclerosis, and hypertension (Yang,*et al*., 2016).

Materials and Method

Study subjects

After getting approval from Kufa science faculty Ethical committee and permission were taken from Thi-qar health presidency. A retrospective hospital study was conducted on 280 CVD patients during 2018 attending to Heart Medical Centre of Thi-qar province which divided into : 200 patients with cardiac catheterization (diagnostic catheters, therapeutic catheters, both) and 80 patients who underwent surgery including Coronary artery bypass grafting (CABG), cardiac valves, and heart defects. The diagnosis based on clinical examinations under supervision of physicians. Members of all groups were informed about the aim of the study and obtained verbal acceptance before taking samples.

A total of 20 healthy subjects volunteer were included as controls group with age range between (25-65) years for cytokines estimation.

Sample Collection

Five milliliter of blood were collected from each subjects by using disposable syringes, then 2 ml was placed in gel tubes allowed to clot at room temperature, centrifuged at 3000 rpm for 10 minutes and sera were dispensed into 3 Eppendorf-tubes, numbered and stored at -20 ºC until used

for measuring, IL-6, TNF and TLR-4 by ELISA technique by using Elabscience kit \ USA

The following samples were excluded: samples from individuals who had received antibiotic therapy; patient not fill all information or correct sample and sample from patients with other type of chronic disease such as DM, hypertension.

Bacterial isolation and identification

Swab from cardiac catheter kit (sheath, cord and needle), Catheter Tip (cut about 5 cm from the tip of the upper catheter) and the remaining 3ml of blood were cultured in blood culture bottles and incubated aerobically at 37ºC in BacT/ALERT 3D for (2-7) days, then cultured on the MacConkey agar, Manitol agar and Blood agar plates and incubated at 37C under aerobic condition for 18-24 hour, Gram's stain was used to examine shape and type of staining. While biochemical tests used according to MacFaddin (2000) in addition to use VITEK- 2 system to confirmed identification.

Statistical analysis

Analysis of data was performed by using Statistical Package for Social Science (SPSS) system/ version 17 and Microsoft Office Excel 2007. Results were expressed as mean ± S.D. P-value was considered significant when it is less than 0.05. The analysis of variance (ANOVA) was also applied.

Result

The results showed that positive bacterial culture appear 62(22.2%) in cardiac catheterized patients and 18(6.4%) of cardiac surgery patients, some specimens show poly-microbial growth. So the total isolates reach to 93 isolates, 75(80.6%) of them were G +ve bacteria, the most common pathogen were *S. aureus* followed by *Streptococcus viridans* and *St. pyogenes* and 18 (19.4) were G-ve bacteria, *Enterobacter cloacae* was most common and 200 (71.4%) specimens gave negative results for bacterial growth.

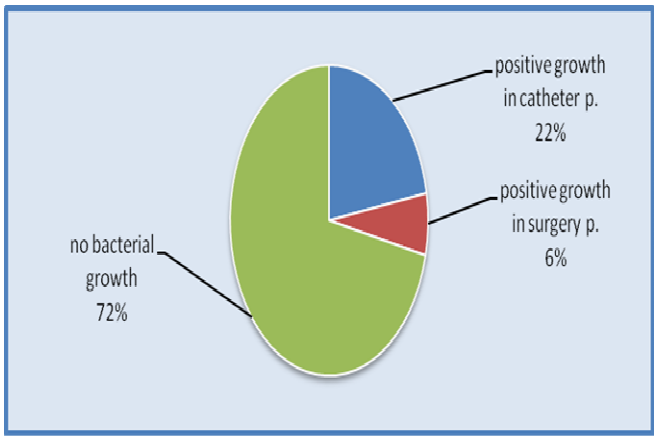

Fig. 1 : Percentage of bacterial growth in patients with catheterization and surgery

Role of IL-6,TNF-α and TLR4 in infected cardiac patients and no infected

The results show that mean serum level of IL-6 were highly increased in cardiac patients in those who suffering from bacterial infection 227.45 pg /ml, then non-infected patient group 150.95 pg/ml as compared with healthy control 51.87 ± 27.70 pg /ml with high significant differences ($p < 0.05$).

The results revealed that statistically significant increase ($P \leq 0.05$) in TNF-α serum level in patients comparison to healthy control which were (28.16 ± 16.82). Patients with bacterial infection showed a higher significant ($P < 0.05$) increase in level of TNF α (164 ± 48.5 pg/ml) then non- infected patients (138.2 ± 22.82 pg/ml) with no significant different between them.

The serum concentration of TLR-4 was elevated in cardiac infected Patients with highly significant differences ($p<0.05$) in compare with healthy group. The concentration of TLR-4 significantly increased in infected patients 17.56 ± 2.9 pg /ml, then in non-infected patients group records 14.12 ± 4.02 pg/ml. While, the control group was record 4.15 ± 0.53 pg/ml, Table (1).

Table 1 : IL-6 ,TNF-and TLR-4 levels in the cardiac patients group and healthy control

Parameter Group	IL_6 (IU/ml) Mean ± SD	TNF_α(IU/m) Mean ± SD	TLR_4(IU/ml) Mean ± SD
Patient with bacterial infection N(40)	227.45 ± 79.71[a]	164 ± 48.50[a]	17.56 ± 2.97[a]
Patient without bacterial infection N(30)	150.95 ± 34.20[b]	138.2 ± 22.82[a]	14.12 ± 4.02
Control N(20)	51.87 ± 27.70[b]	28.16 ± 16.82	4.15 ± 0.53
LSD	42.26	16.70	1.84

Evaluation of IL-6, TNF-a and TLR-4 in cardiac patients with related to age

This study evaluate the concentration of cytokines levels according to age patients revealed that the highest mean of IL-6, TNF-a and TLR-4 serum level of these patients was among the 51-65 which were (295.85; 225.55 and 161.60 respectively pg /ml) followed by the age group of 36-50 years old level of IL6, TNF-α and TLR4 (225.55 ± 27.04[a] , 157± 29.00 and 18.2 ± 1.7 pg/ml respectively). The results were show in Table (2).

Table 2 : IL-6, TNF-a and TLR-4 in cardiac patients with related to age

Parameter group		IL_6(IU/l) Mean ± SD	TNF_a(IU/ml) Mean ± SD	TLR_4(IU/ml) Mean ± SD
Patient 70	(20-35) years.N(22)	161.60 ± 32.43 [bc]	110 ± 17.07 [ab]	12.1 ± 2.0 [a]
	(36-50) years.N(31)	225.55 ± 27.04 [ab]	157 ± 29.00 [bc]	18.2 ± 1.7 [ab]
	(51-65) years.N (17)	295.85 ± 31.89 [a]	225 ± 37.82 [a]	22.7 ± 1.2 [a]
Control. N(20)		51.87 ± 27.70[b]	28.16 ± 16.82	4.15 ± 0.53
L.S.D		**39.63**	**12.38**	**1.65**

Serum Levels of Cytokines on the basis of Operation type

The results of the present study showed a significant increased ($p < 0.05$) in concentration of IL-6, TNF-a and TLR-4 in cardiac catheterized infected patients with mean (290, 210, 22.8 pg/ml respectively) and cardiac surgery infected patients (164, 118, 12.32 pg/ml respectively). All results shown in table (3).

Table 3 : Serum Levels of Cytokines according to Operation type

Parameter Group		IL-6(IU/ml) Mean ± SD	TNF-a(IU/m) Mean ± SD	TLR4(IU/ml) Mean ± SD
Patient with bacterial infection N(40)	Cardiac cath. N (21)	290 ± 35.02[a]	210 ± 31.44 [a]	22.8 ± 3.01 [b]
	Surgery. N (19)	164 ± 26.88 [c]	118 ± 17.23 [c]	12.32 ± 1.07 [a]
Patient without bacterial infection N(30)	Cardiac cath. N (15)	177 ± 35.62 [ab]	178 ± 38.14 [ab]	18 ± 2.12 [e]
	Surgery. N (15)	123 ± 14.82 [bc]	98.71 ± 24.15 [ab]	10.24 ± 1.59 [d]
Control N(20)		51.87 ± 27.70[b]	28.16 ± 16.82	4.15 ± 0.53
L.S.D		27.56	52.04	2.38

Discussion

Bacterial infection may play role in atherosclerosis, coronary artery disease, rheumatic heart disease (Zavareh *et al*., 2016). The longer indwell times of IV catheters are associated with a higher risk of bacterial colonization of cardiac catheters used in humans (Parra-Flores *et al*., 2016). (Bouza *et al*., 2003) they mention that cardiac infection with *S. aureus* is most likely associated with contamination from surgical instruments or from hands of health workers. *St. viridans* are responsible for 40-60% of the endocarditis cases occurring on the normal valves (Giannakopoulos *et al*., 2016).

Cytokines play key roles in protecting the host against bacterial infection by regulating the innate immune response , recruiting phagocytic cells to infection sites (Soderholm *et al*., 2018). In other hand, abnormal release of inflammatory cytokines will lead to systemic inflammatory reactions, aggravate immune function imbalance and further stimulate the inflammatory process that lead to multiple organ failure (Kim *et al.*, 2016).

The data of the study shows that there was a significant difference in levels of cytokines between cardiac patients according to infected or non- infected. The circulating IL-6 levels were increased significantly in all patients with infective endocarditis and significantly higher than patients without infection in compare to control group (Araújo *et al*., 2015). IL-6 elevated in cardiac patients with positive blood cultures higher than patients with negative blood culture (Prendergast, 2006). Similarly (Herlina *et al*., 2016) they shown that IL-6 and TNF –a levels are positively correlated with severity of bacterial infection.

Gram-positive pathogens induced more IL-6 and TNF-α serum levels (Mohamed *et al*., 2007). Maria *et al*. (2013) observed that pro-inflammatory like TNF-a, IL-6 cytokines are elevated in serum IE patients compared to health subjects and higher in staphylococcal and streptococcal IE groups.

Damås *et al*. (2001) illustrated that various microbes play pathogenic roles in cardiomyopathy patients, and such mechanisms could clearly promote enhanced pro inflammatory cytokine such as TNF-α, IL-1 and IL-6 levels in CVD. Also, Infection with certain microbes suggested to be involved in the pathogenesis of atherosclerosis (Becker *et al*., 2001). Microbial antigens may trigger immune activation and increased pro inflammatory cytokines production in patients with CHF (Li *et al*., 2000). Finally, the postoperative period may increase susceptibility to infections because of surgical trauma, catheter and anesthesia-induced transient immunosuppression (Moro *et al*., 2005).

The present study appears that IL-6, TNF- and TLR were significantly difference according to type of operation

Corbi *et al*. (2000) evaluated 31 patients undergoing CABG, they noted that IL-6 concentrations significantly increased with surgery. correlated with study (Supomo, 2019) they observed that IL-6 is increased in patients with surgery, trauma, critical illness and infections.

Elevated IL-6 concentrations have been demonstrated to be associated with impaired hemostasis following cardiopulmonary bypass surgery (Funayama *et al*., 2004). correlated with study (Funayama *et al*., 2004) evaluated 36 patients with cardiac catheterization within 24 hours of infarct onset and found significantly elevated IL-6 values as compared to peripheral vein samples from controls. Cardiac surgery with Coronary artery bypass leads to a systemic inflammatory response with high secretion of IL-6, TNF-a cytokines in post-operative patients and explain the alterations in the serum concentrations of cytokines have important prognostic significance (Roth-Isigkeit *et al*., 1999). Higher plasma levels of TNFα were associated with less favorable outcome after coronary revascularization surgery and may prove useful to utilize TNFα serum levels as a marker for identifying high-risk patients in the future (Suzuki *et al*., 2006)

In current study, there were significant differences according to the type of operation among those infected with bacteria, this study was close to study (Maria *et al*., 2013) they note that Streptococci adhere to the cardiac valves with preexisting endothelial lesions.

Noori *et al.* (2016) showed that in patients with CVD, the serum levels of TNF-α and IL-6 increased compared to control and this increase would be related with hypoxia and remarkable left to right shunt . Interleukin (IL) 1β, IL-6 and TNF-α stimulated by CABG surgery and cardioplegic cardiac arrest that evokes an inflammatory response which may cause temporary organ dysfunction and affect the postoperative course (Altai, 2012). Massoudy *et al.* (1999) mention that the systemic inflammation observed during and after cardiac surgery is related to the secretion of a large number of pro-inflammatory mediators, including interleukin (IL-6, IL-1 B and TNF-α that increased significantly $P<0.0005$ more than two fold in compare with healthy group.

Isigkeit *et al.* (1999) explain in comparative study demonstrated that the serum IL-6, TNF-a increased immediately after aortic cross clamp and reached its peak at the end of surgery in both CABG and heart valve groups.

Ridker *et al*. (2000) In CVD patients, those with high IL-6 levels had .4-fold risk of death compared with patients in the lowest levels, whereas the relative risk associated with high IL-6 among those without CVD was much lower and not significant (Herder *et al*., 2011).

TLR-4 may be constitutively expressed at low levels in normal cells and increase in response to various environmental stresses such as heat shock inflammation, infections by viruses or bacteria, irradiation (Wheeler, 2009).TLR4 plays an essential role in host defense against poly-microbial sepsis by mediating neutrophil migratory/phagocytic functions, and enhanced bacterial clearance (Zhang *et al*., 2014).

Gogos, *et al*. (2000) mention that TLR-4 can recognize molecular patterns associated with a wide range of microbial pathogens in order to initiate transcription of various pro inflammatory cytokines such as IL-1β , TNF-α , IL-,6 and IL-8 . (Cha,*et al*.,2008) showed increased serum level of TNF-α and TLR4 signaling in post-ischemic cardiac dysfunction .

Gram-positive bacteria, such as Streptococci and *S. aureus* interact with Toll like receptor (TLR) type 2 and TLR-4, which is expressed in different cell populations of the innate immunity, including dendritic cells. After this interaction, the dendritic cells secrete IL-6, which is important to induce CD4+ T cells to differentiate into Th1 cells that drive the inflammatory immune response (Oliveira *et al*., 2012). Malley *et al*. (2003) demonstrated that TLR-4 mediates an innate immune response to *St. pneumoniae* through stimulated drives the immune response toward a helper T cell 1 (T_H1) response that lead to activation TNF-a , IL-1 and IL-6.

This study confirmed that there are significant differences in levels of cytokines according to the age of patients, correlated with study by (Klein *et al*., 2006) found that cytokines was significantly associated with age (Myung *et al.,* 2016) the level of pro inflammatory cytokines increases with age and founded that older patients have higher cytokine levels.

Young and middle aged patients revealed positive association between IL-6 levels and the presence of coronary calcification and suggests that elevated IL-6 levels were a strong and predictor of clinically silent CVD (House *et al* ,1990) .

Bruunsgaard *et al*. (2000) illustrated that male were more than female in cardiac catheterization patients and the age more than 40 were more frequently as well as the serum levels of IL-6 and TNF-alpha at the acute phase were significantly higher in patients than in control subjects. The incidence of symptomatic coronary heart disease (CHD), myocardial infarction (MI), and sudden cardiac death increases with a higher life expectancy and an ever increasing number of persons older than 65 in the total population (Shi *et al*., 2016). Finally Huber et al. analyzed the postoperative quality of life in 136 patients between 40 - 80 age and observed that long term survival after cardiac surgical procedures in younger patients than in elderly patients.

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