Serum Interleukin-6 and Highly Sensitive C-Reactive Protein as Inflammatory Biomarkers in Atrial Fibrillation Patients

HELMY H. ELGHAWABY, M.D.†; KARIM S. MASHHOUR, M.D.‡; HAMDY M. SABER, M.D.§ and DOAA EL-GOHARY, M.Sc.∥
The Department of Critical Care, Faculty of Medicine, Cairo* and Beni Suef** Universities

Abstract

Introduction: Atrial fibrillation is associated with increased C-reactive protein level and other acute phase reactant (fibrinogen level, interleukin 2, 6, 8, and others), which raise the question of the role of inflammation in this condition?

Aim of the Work: To assess the relation between atrial fibrillation and serum inflammatory biomarkers (IL-6 & Hs CRP).

Study Design: The study included thirty AF patients. They were subjected to laboratory analysis of their serum IL-6 and Hs CRP.

According to AF duration they were subdivided into two subgroups:
• Recent onset AF subgroup.
• Long standing persistent AF subgroup (AF has lasted for 1 year).

Results: There was a positive correlation of IL-6 & Hs CRP serum levels to the duration of AF (p<0.001 & p<0.001) respectively. There was a significantly higher level of serum interleukin 6 in the persistent AF subgroup denoting a statistically significant difference between this group and the recent onset subgroup (p=0.007). Meanwhile there was no significant difference regarding serum Hs CRP between the recent onset AF subgroup and the persistent AF subgroup (T0.245). There was a significant difference between the two subgroups as regarding left atrial diameter (p=0.031). In the recent onset subgroup left atrial diameter ranged from 41 to 47mm with mean 44.5mm, ±2.5. In the persistent subgroup left atrial diameter ranged from 44 to 71mm with mean 50.3mm, ±6.2mm. There was a significant relation as regarding the incidence of stroke and increased serum level of IL-6 (p=0.001). Serum IL-6 in patients without stroke ranged from 12 to 101pg/ml, with mean 424.5pg/ml, ±313.2 while serum IL-6 in patients with stroke ranged from 980 to 1320pg/ml with mean 1156.7pg, ±110.4.

Conclusion: Interleukin 6 and highly sensitive CRP biomarkers, were found to be good prognostic factors for atrial fibrillation.

Key Words: Atrial fibrillation — C-reactive protein — Interleukin-6.

Introduction

ATRIAL fibrillation (AF) is the most common arrhythmia in clinical practice. AF is defined electrophysiologically as wavelets propagating in different directions causing disorganized atrial depolarization without effective contraction Manifested in the surface ECG by 'absolutely' irregular RR intervals and no distinct P waves, and the atrial cycle length (when visible), i.e. The interval between two atrial activations, is usually variable.

The AF affecting more than 2.3 million people in US, this number increases dramatically with age and is seen in as high as 9% of individual by the age of 80 years. In high risk patients the thromboembolic stroke risk can be as high as 9% per year and is associated with two fold increase mortality.

Many theories were established to discuss etiology of AF. These theories focused on the presence of multiple re-entrant circuits (originating in the atria) that are asynchronous and conducted at various velocities through tissues with various refractory periods.

Another potential mechanism for description of AF is rapidly firing atrial activity in muscular sleeves around the pulmonary veins Ostia.

Much attention has been devoted in the last few years to assess the cascade of inflammation in AF. The contribution of the inflammatory cascade to the onset of AF is suggested by the incidence of AF in post operative cardiac surgeries, a state of inflammatory process.
Studies had already correlated elevation of CRP in healthy individuals to an increased future risk of cardiovascular disease, cerebrovascular events and peripheral arterial disease. Elevation of CRP, IL-6 might also contribute to generation and perpetuation of AF, as evidenced by marked inflammatory infiltrates, myocyte necrosis and fibrosis found in atrial biopsies of patients of Lone AF [5].

**Aim of the work:** To assess the relation between atrial fibrillation and serum inflammatory biomarkers (IL-6 & Hs CRP).

**Patients and Methods**

The study included forty randomized Egyptian patients who were admitted to the critical care unit in El-Kasr El-Aini Hospital, Cairo University or followed-up in outpatient cardiology clinic in El-Helal Hospital from March to September (2010). Patients who had ECG diagnosis of AF were included and signed consents were taken after approval of the ethical committee. Patients with acute coronary syndrome, recent onset febrile illness, recent surgery, renal and hepatic failure, malignancy and any collagen or autoimmune disease were excluded from the study.

The forty patients were divided into two groups:

**Study group:**

This included thirty patients who had AF. Those patients were subjected to laboratory analysis of their serum IL-6 and Hs CRP.

The study group was further subdivided into two subgroups according to the duration of atrial fibrillation, the recent onset AF subgroup and the long term persistent AF subgroup (AF has lasted for year upon enrollment in the study).

**Control group:**

Included ten cases with normal sinus rhythm. They were also subjected to laboratory analysis of their serum IL-6 and Hs CRP.

All study population was subjected to the following:

Full and careful history, clinical examination and 12 lead surface ECG.

**Echocardiography:** It was done using transthoracic echocardiography with M mode, two D mode and Doppler study.

**Laboratory tests:** Patients should fast for at least 12 hours and they should not take aspirin for at least one week. Then two venous samples were withdrawn for serum IL-6 performed by ELISA (Enzyme linked immunosorbent Assay) technique and the other for serum CRP. They were both measured by high sensitivity latex particles turbidimetric assay.

**The statistical methods:**

Data were statistically described in terms of range, mean±standard deviation (±SD), median, frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5 Accuracy was represented using the terms sensitivity and specificity.

A probability value (p-value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY and USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

**Results**

**Demographic data:**

This randomized study included forty Egyptian cases, thirty of them had AF (the study group) and the remainder ten had normal sinus rhythm (the control group). The control group was all male patients, while the study group consisted of 23 males, 7 females. The mean age of control group was 35.8±7.5, while in the study group was 46.5±9.6. In the study group 8 patients were diabetic, 4 patients hypertensive, 2 patients had both and 16 patients were smokers, compared with the ten healthy control group subjects.

Regarding underlying cardiac pathology the study group consisted of 5 patients who had IDCM, 5 had IHD, 3 had MVP, 3 had rheumatic mitral valve disease.

**A- Bio markers data:**

**Serum IL-6 levels in both groups:**

There was a significant difference between the two groups as regarding serum interleukin-6 ($p<0.001$). In the study group serum interleukin-6 ranged from 310 to 1320pg/ml with mean 700.7pg/ml, ±305.6. In the control group serum interleukin-6 ranged from 12 to 65pg/ml with mean 35.2pg/ml, ±16.7.

**Hs CRP levels in both groups:**

Also there was significant difference between the two groups as regarding serum highly sensitive
C-reactive protein (p<0.001). In the study group serum C-reactive protein ranged from 3.0 to 98.0mg/1 with mean 25.4mg/1, ±26.9. In the control group serum C-reactive protein ranged from 2.0 to 6.0mg/1 with mean 3.3mg/1, ±1.2.

- IL-6 and AF duration:

In the persistent subgroup serum interleukin 6 ranged from 390 to 1320pg/ml with mean 747.3pg/ml, ±301.4 that was significantly higher than the recent onset subgroup as serum interleukin 6 ranged from 310 to 480pg/ml with mean 397.5pg/ml, ±69.5 (p=0.007) (Fig. 1).

- Hs CRP and AF duration:

Serum CRP in the recent onset subgroup ranged from 5.6 to 21.0mg/1 with mean 12.5mg/1, ±7.7 and that was lower than the persistent AF subgroup serum CRP which ranged from 3 to 98.0mg/1 with mean 27.4mg/1, ±28.3 but these changes do not reach statistical significance (p=0.245).

- Correlation between both IL-6 and Hs CRP and AF duration:

There were a positive significant correlation between duration of atrial fibrillation and serum interleukin 6 and highly sensitive C-reactive protein. As with increasing duration of AF, serum IL-6 and Hs CRP also increased, with (r=0.862, 0.566) for IL-6 and Hs CRP respectively (Fig. 2).

- IL-6 and stroke:

There was significant relation between the incidence of stroke and increased serum level of IL-6 in the whole study population (p=0.001). Serum IL-6 in patients without stroke ranged from 12 to 1010pg/ml, with mean 424.5pg/ml, ±313.2. While serum IL-6 in patients with stroke ranged from 980 to 1320pg/ml with mean 1156.7pg/ml, ±110.4 (Fig. 3).

- Hs CRP level and stroke:

There was significant relation between the incidence of stroke and increased serum level of CRP in the whole study population (p=0.001). Serum C-reactive protein ranged from 2.0 to 48.0mg/1 in cases without stroke, with mean 11.5mg/1, ±10.3. While in cases with stroke serum C-reactive protein ranged from 30.0 to 98.0mg/1 with mean 67.1mg/1, ±32.9 (Fig. 4).
B- Echocardiographic data:

- Left atrial diameters between the two study groups:

  There was significant difference between the two groups as regarding left atrial diameter (p<0.001). In the study group left atrial diameter ranged from 41 to 71mm with 49.5mm, ±6.1. In control group left atrial diameter ranged from 31 to 40mm with mean 35.2mm, ±2.9.

- Left atrial diameters between the subgroups:

  There was a significant difference in between the two subgroups as regarding left atrial diameter, (p=0.031). In the recent onset subgroup left atrial diameter ranged from 41 to 47mm with mean 44.5mm, ±2.5. In the persistent subgroup left atrial diameter ranged from 44 to 71mm with mean 50.3mm, ±6.2mm. As AF became chronic and persistent, left atrial diameter increased.

Discussion

Many theories were established to discuss etiology of AF. These theories focused on the presence of multiple re-entrant circuits (originating in the atria) that are asynchronous and conducted at various velocities through tissues with various refractory periods [2].

Much attention has been devoted in the last few years to assess the cascade of the inflammation in AF. The contribution of the inflammatory cascade to the onset of AF is suggested by the incidence of AF in post operative cardiac surgeries, as a state of inflammatory process [4].

Studies had already correlated elevation of CRP in healthy individuals to an increased future risk of cardiovascular disease, cerebrovascular events and peripheral arterial disease. Elevation of CRP, IL-6 might also contribute to generation and perpetuation of AF, as evidenced by marked inflammatory infiltrates myocyte necrosis and fibrosis found in atrial biopsies of patients of Lone AF [5].

In our study we are trying to assess relation between atrial fibrillation and serum inflammatory biomarkers especially serum IL-6 and highly sensitive CRP as high risk diagnostic and prognostic factors. All study population was subjected to laboratory analysis of their serum IL-6 & Hs CRP to detect relation between these inflammatory biomarkers and AF (Duration, Left atrial diameter and complication of AF as stroke).

Despite that our study design was not the same like the one done by Tong Liu, et al. [6]. Who were trying to prove the relation between C-reactive protein and interleukin-6 and recurrence of atrial fibrillation after successful electrical cardioversion, but we share the same conclusion that there is inflammatory role in the pathogenesis of AF, its initiation, perpetuation and its recurrence. Their study consisted of 420 patients, serum Hs CRP & IL-6 were withdrawn on day 0 and then after electrical cardioversion, and follow-up period varied between 7 and 140 days. The results were 229 patients in the AF recurrence group who had greater inflammatory cytokines levels compared to the 191 patients in the no AF recurrence group. There was significant relation between inflammatory biomarkers (Hs CRP & IL-6) and recurrence of AF with (p=0.05). This study proved that increased baseline CRP & IL-6 levels were associated with higher risk of AF recurrence after successful electrical cardioversion, this supported that there is an inflammatory role in the pathogenesis of AF, its initiation, perpetuation and its recurrence, and that agrees with our finding that high level of HS CRP & IL-6 correlate with long standing duration of AF.

Our study comes in agreement with Shervin Ziabakhsh-Tabari [7]. Their objectives were to examine the relationship between proinflammatory cytokines, such as Hs CRP & IL-6 and atrial fibrillation after on pump coronary artery bypass grafting (CABG). Fifty-four patients with coronary artery disease were undergoing elective CABG. From 54 patients, 11 patients developed AF after CABG. Preoperative HS CRP and IL-6 levels were higher in patients with AF. The Hs CRP and IL-6 increased after CABG in all patients, but it increased more in the AF group. This study agrees with our work as there was a significant relationship between high levels of IL-6 & Hs CRP and AF in our patients.

Another study of Dernellis and Panaretoue [5] examined the effect of atorvastatin in patients with Paroxysmal AF. Eighty patients were randomized into 40mg ofatorvastatin and or placebo. In the atorvastatin arm, Hs CRP & IL-6 level were lowered, decreased by 2.4 from baseline with (p=0.01) and resolution of Paroxysmal AF was seen in 26 of 40 patients with (p<0.001) at 6 month follow-up. This study further supports the notion that Hs CRP & IL-6 can be considered as risk factors for AF.

In our study there was a positive correlation of IL-6 and Hs CRP serum levels to the duration of
AF with (p<0.001 & p<0.001) for interleukin-6 & Hs CRP respectively. There was also a significant difference in serum interleukin 6 levels between recent onset subgroup and persistent subgroup with (p=0.007). Although, there was no significant difference as regarding serum Hs CRP between recent onset AF subgroup and persistent AF subgroup (p=0.245).

As in Stavroula et al. [8] there was a positive relation between interleukin-6 and duration of AF with p-value 0.02, but there was no relation with Hs CRP and duration of AF. This agrees with our study, as when AF became chronic and persistent serum interleukin 6 levels increased, so IL-6 acts as good indicator of chronicity although we couldn't prove any significant relation regarding AF duration and CRP levels.

In contrast to our results, the study conducted by Watanabe et al. [9] in which Hs CRP and IL-6 was elevated in paroxysmal atrial fibrillation group than in control group with (p=0.003). There was positive correlation of the duration of AF and Hs CRP and IL-6. This means that both Hs CRP and IL-6 act as good indicators of chronicity. That does not go with our study, as the relation is true only for IL-6 and not for Hs CRP.

The results of our study come along with the study done by Kristoffer et al. [10]. Hs-CRP & IL-6 were measured in 56 patients with persistent AF (mean 128 days, range 14-960), 19 healthy volunteers and 19 patients with permanent AF. Patients with persistent AF underwent cardioversion. Patients with permanent AF had significantly higher levels of Hs-CRP & IL-6 than patients with persistent AF (p<0.001). This study concluded that AF patients had elevated levels of inflammatory markers.

Our work agrees with study done by Gregory et al. 1111 they tried to prove that atrial arrhythmias are associated with inflammation. They performed a prospective observational study where Hs CRP and IL-6 levels from the femoral vein and coronary sinus were compared before curative ablation for AF (n 59) and SVT (n 110), follow-up levels were obtained at 1 and 6 months. Peripheral levels of both biomarkers were significantly higher in the AF group. Only those in the AF group had significantly elevated Hs CRP levels & IL-6 with (p=0.0333). Levels of each marker were similar in the coronary sinus and peripheral blood in the SVT group; while in the AF group, both Hs CRP & IL-6 were significantly lower in the coronary sinus than in the periphery (p=.0076 & p=.0021) respectively. Hs CRP was significantly lower in a median of 47 days after AF ablation and remained reduced at second follow-up. No reduction in inflammatory biomarkers was observed after SVT ablation that suggests that atrial fibrillation is strongly related to inflammatory process.

Study of Chung et al. [12] examined serum Hs CRP of 131 patients with atrial fibrillation compared with serum Hs CRP in 71 control group. Serum Hs CRP was higher in AF group than in control group with (p<0.005). Also serum Hs CRP was higher in persistent and permanent AF than in paroxysmal AF patients with (p=0.008). This comes in agreement with our study. This means that with increase duration of AF episodes, the level of inflammatory biomarkers also increases.

Marcus et al. [13] in The Heart and Soul Study, performed cross-sectional analysis of 971 participants who had stable coronary artery disease, 46 of whom had AF. Serum interleukin-6 was significantly higher in AF group than those with sinus rhythm group with (p=0.005), whereas Hs CRP was not, with (p=0.37). This suggested that IL-6 was a unique important mediator in the pathophysiology of AF, so only IL-6 acts as a good predictor of AF, whereas Hs CRP was not. That comes in agreement with our study.

In our work we tried to assess the relation of serum IL-6 and Hs CRP levels and stroke development among atrial fibrillation patients. Mean serum IL-6 was 1156±110.4 in all cases who had stroke and 424.5±313.2 in patients without stroke (p=0.001). The same applies for Hs CRP level, it was 11.5±10.3 in cases without stroke and 67.1±32.9 in patients with stroke (p=0.001), this findings may suggest that serum IL-6 Hs CRP may act as a predictor for high risk patients for stroke development although further investigation may be needed.

Study by Dwayne et al. [14] who undertook a pilot study to determine dates of stroke or death occurring among 77 AF cases with stored plasma samples having initially been obtained during attendance at their AF clinic between “1993-1995”. Plasma IL-6 & Hs CRP were measured respectively. These patients were followed-up for a median duration of 2305 days (7 years). During this period, there were 8 strokes and 22 deaths with high IL-6 level of these patients. High level of IL-6 remained significant predictor of stroke or death (Even after adjustment for age with (p=0.007).
Trends toward increased risk with high plasma Hs CRP did not reach statistical significance with (p=0.06). These results come in agreement with our current study regarding only levels for serum IL-6, as our study had both biomarkers with statistical significance as prognostic factors for stroke.

Our study agrees with the work done by Watanabe et al. [9], they investigated 50 patients with paroxysmal AF, split into 2 groups on the basis of duration of AF. Short paroxysmal AF was less than 30 days, and long paroxysmal AF was more than 30 days. Control group consists of 20 patients free with normal sinus rhythm Hs CRP and IL-6 were elevated in paroxysmal AF groups than in control group with (p=0.003). Long paroxysmal AF had higher Hs CRP and IL-6 levels than in short paroxysmal AF. Both short paroxysmal AF and long paroxysmal AF had larger LA diameter than control group. This means that there was positive relation between serum Hs CRP and interleukin-6 as inflammatory biomarkers and duration of AF, which is the same for our study. Long paroxysmal AF had larger LA size than of short paroxysmal AF. This means that there was positive relation between left atrial diameter and AF duration. There was positive relation between serum Hs CRP and IL-6 and left atrial diameter. This also comes in agreement with our study. Elevated Hs CRP and IL-6 indicates that inflammation has a role in the development of AF and LA remodeling.

Finally:

As regarding the non significant increase of Hs CRP in contrast with statistically increased IL-6 for AF duration, there was no available data in literature to explain this. But our opinion suggests that there might be difference regarding the effect of acetyl salicylic acid (aspirin), which was stopped 12 hours before the blood samples withdrawal on both inflammatory biomarkers with a delayed inhibitory effect on Hs CRP.

Conclusion:

Our study supported that atrial fibrillation is an inflammatory process, which is responsible for initiation and perpetuation of AF and responsible for left atrial remodeling. Inflammatory mediators as IL-6 & Hs CRP act as high risk and prognostic factors for AF.

Limitation of the study:

Small and heterogeneous pathology in the study group. Selecting one single etiology for atrial fibrillation in the study group will enforce the value of the results.

References