Medico-legal and Serological Study of the Role of C- Reactive Protein and Anticholesterol Auto-antibodies in the Diagnosis of Ischemic Heart Disease

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ABSTRACT:

BACKGROUND:

No previous Iraqi study was done on the role of C - reactive protein (CRP) and anticholesterol autoantibodies (ACHA) in the diagnosis of ischemic heart disease (IHD) especially from the medico-legal point of view.

AIMS OF THE STUDY:

To determine the role of CRP and ACHA in the diagnosis of IHD particularly myocardial infarction (MI) with special attention to the medico- legal aspect.

METHODS:

Forty four lived patients from Al- Kadhimiya hospital and 20 cadavers in medico-legal institute of Baghdad were included in this study, in addition to 18 apparently healthy persons and 3 cadavers as controls. A number of risk factors were studied such as age, sex, smoking, and others. CRP and ACHA detected and estimated in the sera of the lived and dead patients. Histopathological examination was done on cardiac tissue specimens taken from the cadavers.

RESULTS:

Patients with anterior MI have higher CRP values than in patients with other types of MI. ACHA of IgM type was higher in controls than in lived and patients, while that of IgG was higher in lived patients as compared with dead patients and controls.

CONCLUSION:

Elderly males are affected more by MI. CRP is elevated in acute coronary syndrome. ACHA are present in healthy individuals, but high in CAD.

KEY WORDS: CRP, ACHA, myocardial infarction, medico-legal, serological.

INTRODUCTION:

The coronary artery circulation normally supplies blood flow to meet demand of the myocardium as it labors under a widely varying work load. An imbalance between coronary blood flow and myocardium oxygen consumption (demand) can precipitate ischemia which frequently manifests as angina pectoris. When the imbalance becomes extreme myocardial infarction (MI) may result. Congestive heart failure, and arrhythmia are the major complications of MI, and arrhythmia is probably the major cause of sudden death in ischemic heart disease (IHD). [1] Cardiac pathologists claim that at least 80% of the normal lumen of the coronary artery must be lost before myocardial necrosis occurs. [2, 3]

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In more than 90% of cases, the cause of myocardial ischemia is reduction in coronary blood flow due to atherosclerosis. [4] Coronary heart disease is the most common form of heart disease and the single most important cause of premature death in the developed world, especially in developed countries.[5]

A number of risk factors of atherosclerosis have been reasonably well established on the basis of their relation in epidemiological studies to the clinically manifested disease. These include hyperlipidemia, hypertention, cigarettes smoking, male sex, diabetes mellitus, family history, haemostatic factors, physical activity, obesity, alcohol, other dietary factors, and may be mental stress. [6] MI causes a detectable rise in the plasma concentrations of enzymes and proteins that are normally concentrated within cardiac cells. Plasma biomarkers that are widely used in the detection of MI are creatine kinases (CK), a more sensitive and cardio-specific isoform of this enzyme is (CKMB). [5] Prospective epidemiological studies have shown a strong and consistent association

between clinical manifestation of atherosclerotic disease and systemic marker of inflammation, including white blood cells count, and various haemostatic proteins that are also acute phase reactants such as fibrinogen- activator or inhibitor type-1(Von willbrand factors). [7]In addition there are other serum markers of inflammation that provide an avenue of insight into the pathophysiology of atherosclerosis and its complications, like high-sensitivity testing for C-reactive protein (hs-CRP), soluble forms of leukocyte adhesion molecules, such as soluble intercellular adhesion molecule-1 (sICAM1). [8, 9] These distal markers may reflect the consequences of elevated levels of proinflammatory cytokines, for example: interleukin-6 (IL-6) which probably provokes the augmented expression of C-reactive protein (CRP) gene in the liver. Cytokines such as tumor necrosis factor-& (TNF-&) or IL-1 isoforms can in turn stimulate the expression of IL-6 and of the leukocyte adhesion molecules, such as ICAM-1. [10] Alving and Swartz, *et al*., found that sera of virtually all healthy subjects contain IgM and IgG autoantibodies against cholesterol (ACHA). They found that the serum concentration of ACHA was significantly lower in patients with peripheral atherosclerosis and cerebrovascular disease but significantly higher in patients with CAD compared with age-matched healthy controls. They are natural antibodies, and their levels are independent of the amount of cholesterol in various lipoprotein fractions. These antibodies are different from anti-phospholipids antibodies. [11] These findings were supported by Horvath *et al*., when they found ACHA-IgG levels were lower in patients with peripheral occlusive atherosclerosis and ischemic stroke but considerably higher in CAD patients than in the age-matched controls. [12] After death the diagnosis is made by macroscopical and microscopical features in the affected myocardium and this can have profound medico-legal implications. [2]

MATERIALS AND METHODS:

1. SUBJECTS:

A- Patients group: Forty four survived patients (35 `males and 9 females) their age ranged from 40 to 65 years old with acute and sever chest pain admitted to the Cardiac Care Unite (CCU) in AL-Kadhimyia Teaching Hospital in Baghdad from the 1st of April 2005 to the 30th of September 2005 were included in this study. The diagnosis was based on clinical presentation and history of 1HD, which was confirmed by ECG, cardiac enzymes and ECHO, to be either unstable angina or MI, 2 of them died during this study. Twenty autopsies were done to persons died suddenly due to cardiac ischemia in the medico-legal institute of Baghdad. The selection of these autopsies depended on the presence of completely occluded coronary artery.

We took specimens from the suspected sites of the myometrium to confirm the diagnosis by histopathological examination.

B-Control group: Eighteen apparently healthy individuals with age and sex matched were included as control in addition to three heart tissue autopsy specimens, one of them from a person who died from a road traffic accident, and tow other cardiac tissue were taken from two individuals, died due to head injury (bullet) to be used as control for tissue staining.

2. Baseline data: Baseline data about the lived patients were obtained form routine history and clinical examination, these include: name, age, sex, smoking, hypertension, diabetes, obesity, family history, occupation, chief compliant and duration of pain. Whereas, baseline data about the dead persons were taken from their relatives who were asked by the forensic pathologist, in addition to the information provided in the autopsy requests and reports.

3. Samples: Two mls of blood were taken from each of the 44 survived patients. Blood samples were also taken from the 18 healthy individuals and the 20 dead persons. The samples were collected in plain tubes and allowed to clot and then centrifuged for 10 minutes at 3000 rpm. Sera were aliquoted and frozen at – 40C till their use in evaluation of cholesterol – specific antibodies (1gM and 1gG) and CRP. Heart autopsy specimens were taken from 20 dead persons and 3 controls, fixed in formalin and embedded in paraffin. Tissue blocks were sectioned (4*u* thickness) and stained with haematoxyline and eosin.

4. Histomorpholgical diagnosis: According to histopathological features, the tissue sections were classified into (A) no changes, (b) early ischemic changes, (c) advanced infarction and (d) old infarction.

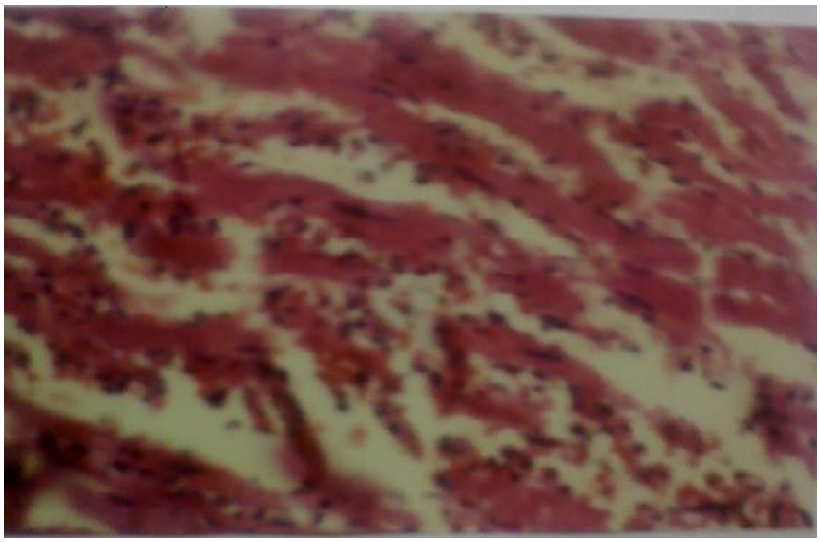
5. Enzyme-Linked Immuno-Sorbent Assay (ELISA): ELISA was used for determination of cholesterol-specific antibodies. Direct ELISA was involved at the first absorption of antigen on the wells surfaces of microtiter plates, serum samples from the tested blood were added, and then antigen-

specific antibodies present in the sample would bind to the antigen. Unbounded (non specific) antibodies were washed away; secondly the antibodies (conjugated) were then bounded to the specific antibodies that already bounded to the antigen. The excess of conjugate is washed away, and the substrate solution was added to each well. The amount of color developed as the substrate was acted upon by the enzyme of conjugate was directly proportional to the amount of antibodies in the serum.

6. Statistical Analysis: Statistical analysis was performed with the SPSS 10.01 statistical package for social sciences and also Excell 2003. Data analysis was done using chi-sequare test for tables with frequencies while we use student t test for tables with means and standard deviations and ANOVA if we have more than two groups. P-value of < 0.05 was used as the level of significance. Descriptive statistics for the clinical and for serological data were done using the percentage, mean and SD (standard deviation).

RESULTS:

1. Clinical and histopathological distribution of the patients: Sixty four patients with IHD were included in this study, 44 lived and 20 were dead as a result of ischemia. Lived patients were classified clinically into anterior MI (n=9), anterolateral MI (n=9), inferior MI (n=14), and unstable angina (n=12). Seventy two percent of patients were MI, and 28% were unstable angina. The frequency of patients with MI is higher than the frequency of patients with unstable angina. The dead persons were classified according to histomorphplogical changes into four main groups including no changes group (n=4), early ischemic changes group (n=6), advanced infarction group (n=4), and old infarction group (n=6). Photograph (1) shows the microscopical histopathological changes of early myocardial infarction (the details are mentioned in the paragraph of discussion).



Photograph (1): Histopathological changes of early myocardial infarction (H&E stain x 400)

2. Age distribution: The mean age of IHD patients was (57.26563 $\pm$ 9.253472) years old, and ranges from 40-73 years old. The mean age of MI lived patients group was (59.56667$\pm$10.89031) years old and it was higher than that of the unstable angina lived patients group which was (45.08333$\pm$6.570711). The mean age of the dead persons was (65.38095$\pm$10.21996) years old and it was higher than that of MI and unstable angina as shown in (figure 1). There were no significant differences in the frequency of the patients in each group ($p<0.179485$).

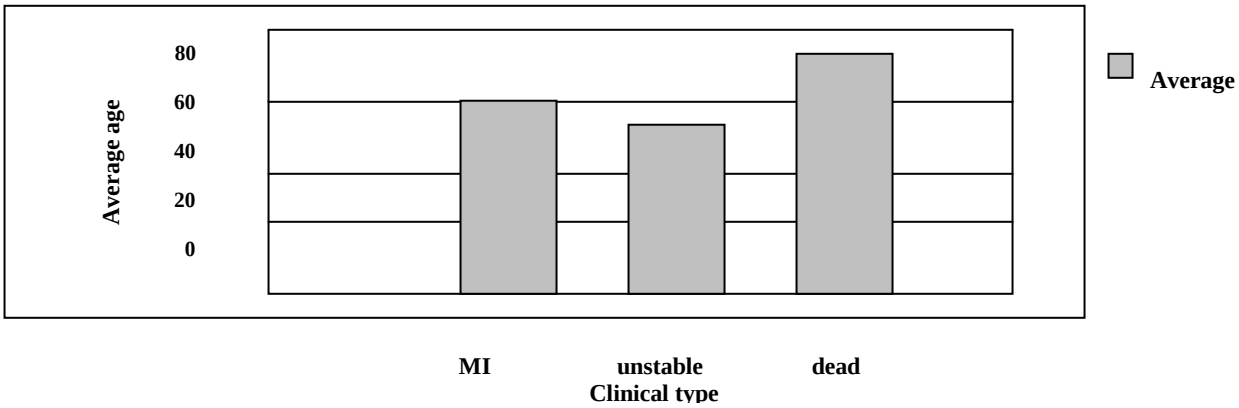


Figure (1): The average age of lived patients (MI & unstable angina) and the dead Patients group.

3. **Sex distribution:** Fifty five (86.15385%) of the patients were male and 9(13.84615%) were females (figure 2). The male to female ratio was (6:1). The observed frequencies in male group were higher than the expected. There were statistically no significant differences between the frequency of the patient in male and female group as it was revealed by chi-square test ($p<0.8415$).

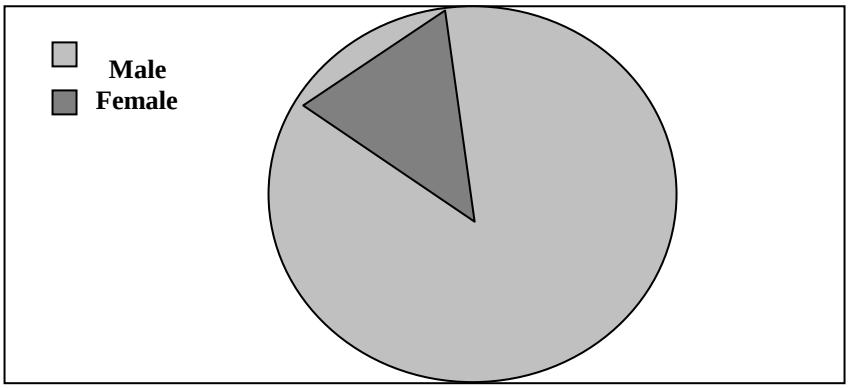


Figure (2): Sex distributions of 64 patients (lived and dead)

4. **Risk factors:**

(a) Smoking: Forty seven (73%) of the total number of the patients were smokers. Thirty one (66%) of the smokers were from the lived patients while 16 (34%) were` from the dead persons. Chi-square test reveals a highly significant differences between the frequency of the patients in the smoker group when compared with the control group ($p<0.0001$).

(b) Hypertension: Twenty seven (42%) of the patients were hypertensive and chi-square reveal a significant differences between patients and control group ($p<0.05$), but there were no significant differences between the frequency of the lived and dead patients in hypertensive group ($p<0.6547$).

(c) Diabetes: Twenty one (33%) of the patients were diabetics, and statistically there were no significant differences between the frequency of the lived and dead patients ($p<0.3623$), but there were significant differences between the dead and the controls ($p<0.001$).

(d) Obesity: Four (6%) of the patients were obese. Chi-square test reveals a significant differences between patients and control group ($p<0.05$) but there were no statistically significant differences between lived and dead patients frequency within the obese group.

(e) Family history: Twenty one (33%) of the all the patients had a family history of IHD, chi-square

reveals no statistically significant differences between patients and controls (p<0.4096), and between lived patients and the dead (p<0.8625).

5. Response to treatment:

The patients in this study were classified according to their response to treatment into complete response 31%, partial response 23%, patients with progressive disease 11%, and 35% were dead patients.

6. Serological tests:

(a) C-Reactive Proteins CRP: The results reflect that patients with anterior MI have a higher CRP levels than anterolateral MI, inferior MI, and unstable angina (see figure 3), but statistically there were no significant differences between the groups (p<0.430588).

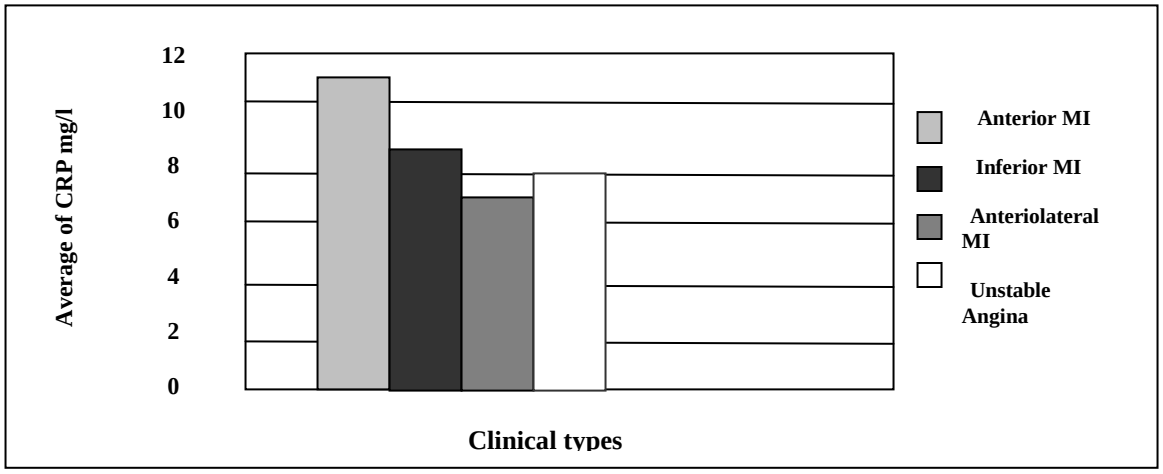


Figure (3): Average of CRP levels in each clinical type of lived patients

(b) Anti-cholesterol autoantibody (IgM): ANOVA analysis reflects significant differences between patients and controls (p<0.00193), The anti- cholesterol autoantibody IgM was higher in controls as compared with dead and lived patients (see figure 4).

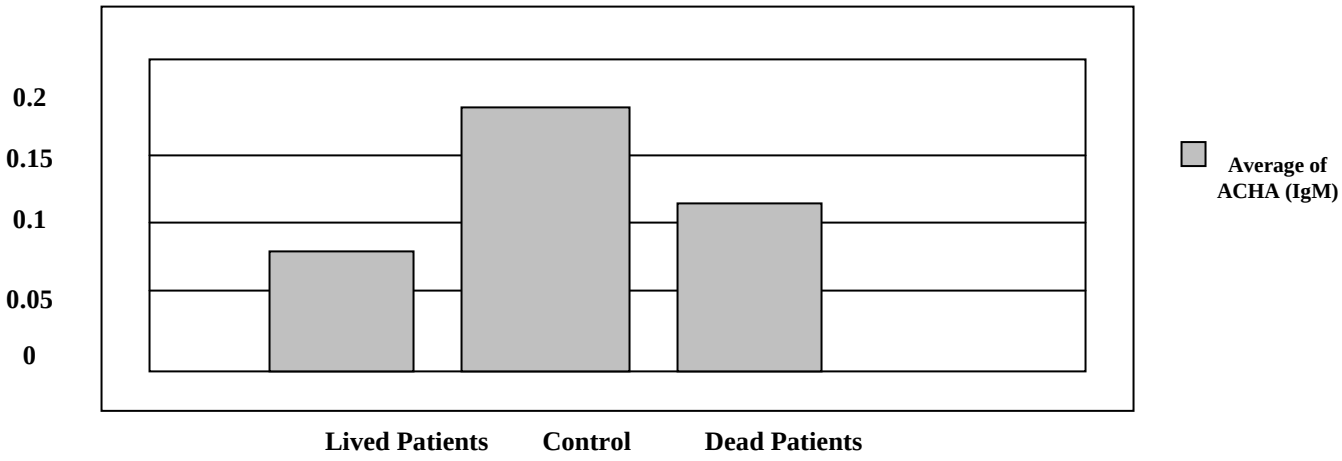


Figure (4): Average of IgM level in controls, Lived and dead patients.

ANOVA test revealed that the higher level of IgM was in unstable angina and the lower level was in anterolateral MI (see figure 5). Statistically there were no significant differences between the patient groups (p=0.767771).

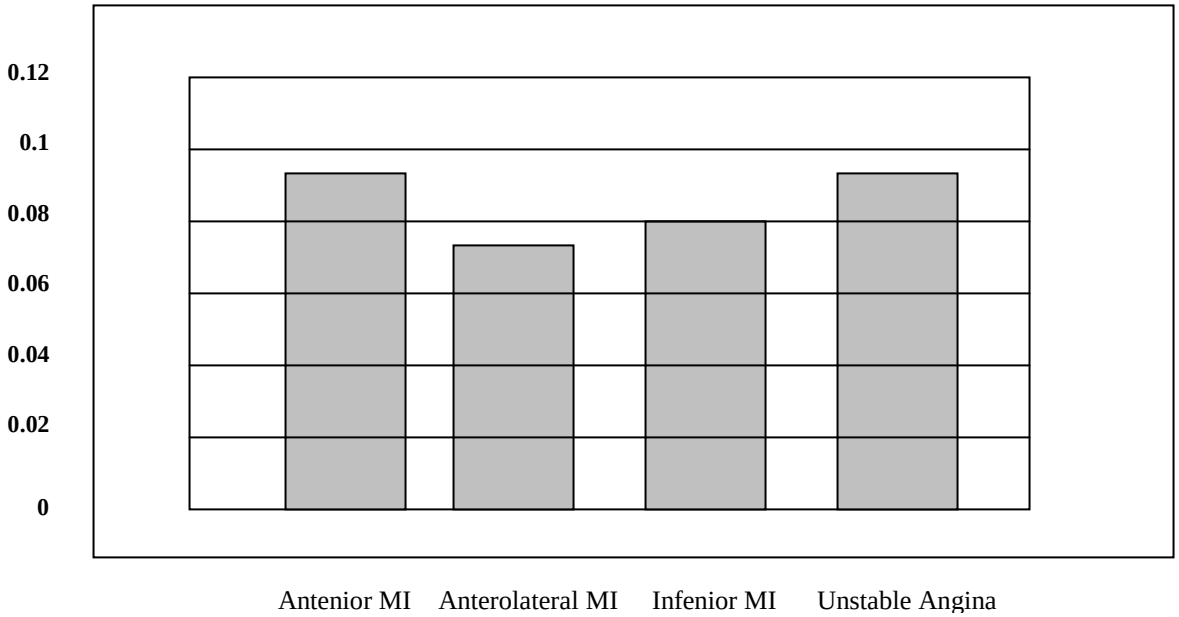


Figure (5): Average of IgM level in each clinical type of lived patients.

(c) Anti-cholesterol autoantibody (IgG): ANOVA test reflects that the levels of IgG anticholesterol autoantibodies were higher in lived patients as compared with the dead patients and controls (see figure 6), but also there were no significant differences between the groups ($p<0.738351$).

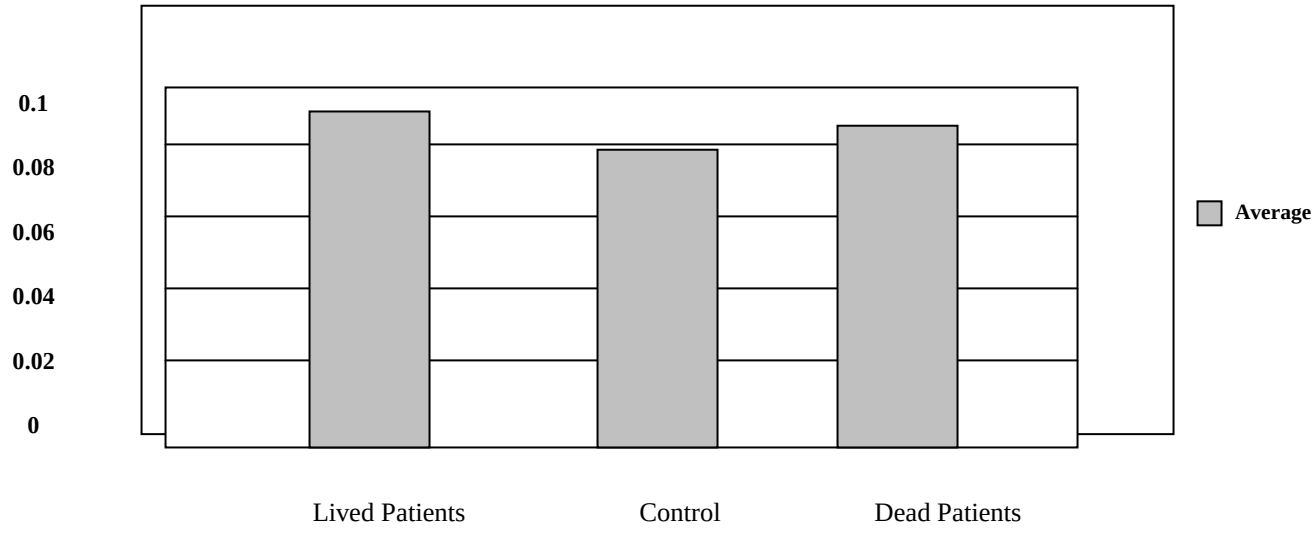


Figure (6): Average of IgG levels in controls, Lived and dead patients.

The observed data showed that the highest level of IgG anticholesterol autoantibodies in unstable angina followed by the anterior MI patients group then inferior and anterolateral MI patients group. There were no statistically significant differences among patients groups ($p<0.26098$). (See figure 7)

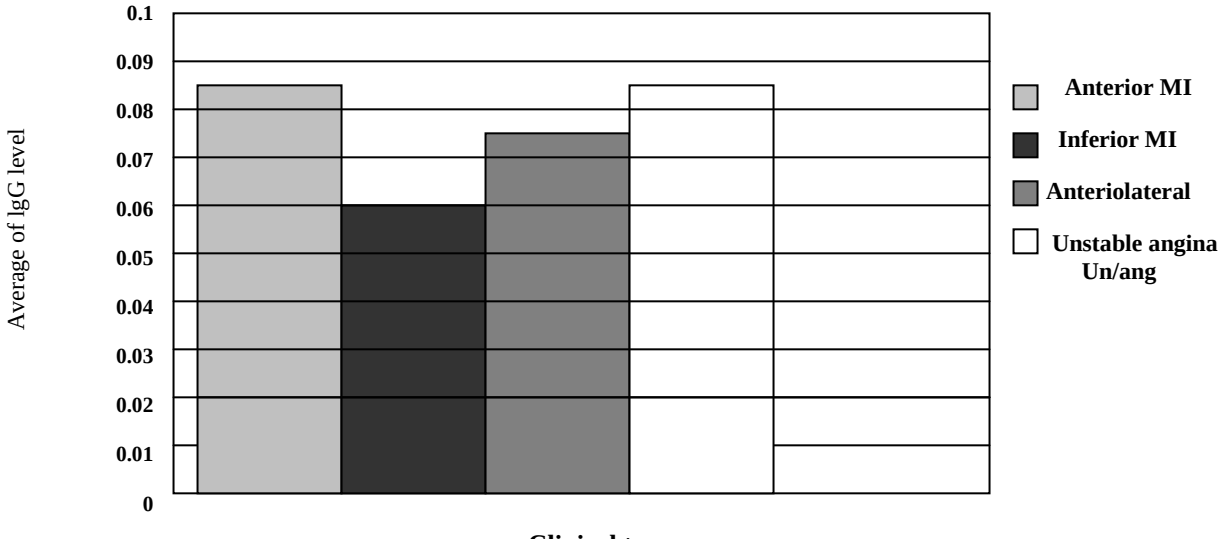


Figure (7): Average of IgG levels in each clinical type of lived patients.

DISCUSSION:

In recent years, substantial evidences indicate an important role for inflammatory mechanisms in the pathophysiology of cardiovascular diseases although most of these evidences were obtained from studies done on laboratory animals (mostly rats and mice) as well as primary cultures of cardiac cells. However, immune system and immune response of the rat or mouse may differ from that of the human and the validity problem which associates with previous in-vitro studies like the problem of metabolites accumulates in the culture medium which could influence the sensitivity of endothelial cells. Upon that we tried to focus on immunopathogenesis of the acute MI by this prospective study which was done on human cardiac tissue sections. We agreed with a previous study which stated that the potential role of inflammation in mediating pathological changes associated with IHD has not been adequately investigated. [13] Many obstacles we faced in this study including; the cardiac tissue biopsies from lived individuals were impossible, however, autopsy specimens obtained from patients died from cardiac attack as a result of arrhythmia or ventricular fibrillation may revel no pathological and molecular changes. Those who die from sudden, severe and massive myocardial infarction may have no time to reveal any new pathological changes (death had occurred within a few hours after the onset of the symptoms). And those who die from complicated old MI; show only old changes. Moreover, the number of references that studied acute MI in human was few and we could not find any Iraqi reference to compare our result with. To overcome such obstacles we selected a group of dead patients from cardiac attack and proved to have MI by autopsy examination in the medico-legal institute in Baghdad. In such group the results of serological examinations of the patients were compared with age and sex matched group of apparently healthy individuals. The mean age of IHD patients in our study was (57.2) years old and the range was from (40-73) years and that agrees with a previous study done by Garas et al in (2006) who stated that most of the patients who developed CHD were around and older than (60) years, [14] Whereas the mean age of the dead patients in our study was (65.38) years old; a result which agreed with a previous study which stated that the elderly people tend to have a higher rates of morbidity and mortality from their infarcts. Also in our study the frequency of MI patients is higher than in unstable angina and that goes with the study of Thom et al in (2006) who observed that the prevalence pf MI was higher among (40-74) year's old individuals than unstable angina. [15] The frequency of male patients was higher than that of females in our study (see

figure 2) and that agrees with a previous study which stated that premenapausal women are generally protected from the manifestations of IHD because of the protective effects of estrogen. [16] Forty seven (73%) of total number of our patients were smokers and 17 (27%) were none smokers. There was a highly significant difference between patients and control group (P<0.001). Our results may indicate an association between smoking and IHD; A result that agrees with other epidemiological studies which showed that the prevalence of smokers within IHD patients was higher than that of the normal population. [15] Twenty seven (42%) of our patients were hypertensive, and 37(58%) were none hypertensive. A comparison between the frequency of the patients and the frequency of the control within hypertensive group revealed a significant difference, and the frequency of the patients within hypertensive group patients was higher than that in control group ($p<0.05$). Our results agree with Garas el al in 2006 who considered hypertension as modifiable risk factors for IHD. [14] The incidence of hypertension among our patients (42%) is lower than what was reported in an epidemiological study done in 2006 which revealed that about 69% of people who have a first heart attack, 77% who have a first stroke, and 74% who have CHD have blood pressure higher than 140/90 mm Hg. [15] Twenty one (33%) of the patients were diabetic, 43(67%) were none diabetics. A comparison between the frequency of the patients and the frequency of the controls within diabetic group revealed a significant difference, and the frequency of the patients within diabetic group patients was higher than that in control group ($p<0.05$). Our results agree with a study done by Garas el al in 2006 who considered diabetes mellitus as a modifiable risk factor for IHD and atherosclerosis. [14] The incidence of diabetes mellitus (DM) among our patients was lower than what had been reported in an epidemiological study done in 2006 which revealed that about 55% of the IHD patients had DM. [15] Four (6%) of the patients were obese, and there was a significant difference between patients and control group ($p<0.004$). A comparison between the frequency of the patients and the frequency of the control within obese group revealed a significant difference, and the frequency of the patients within obese group patients was higher than that in control group ($p<0.05$). Our results agree with a study done by Garas el al in 2006 who considered obesity as modifiable risk factors for ischemic heart disease and atherosclerosis. [14] The prevalence of obesity among our patients (6%) is lower than what is reported in an epidemiological study done in 2006 which revealed that about 30% of the IHD patients were obese. [15] Twenty one (33%) of the patients had a positive family history of IHD, and there were no statistically significant differences between the frequency of the patients with positive family history and those with negative family history. Such a result may not agree with a previous study done by Garas el al in 2006 who considered family history as a non-modifiable cause of IHD and atherosclerosis. This discrepancy between our result and the result of that study could be due to the smaller size of our patients. [14] In relation to histomorphological features examined by light microscopy using ordinary stain the tissue specimens taken from the dead patients in this study were classified into four main groups:

(a) No changes group (n=4): There were no observed apparent histomorphological findings and that was because their death had happened before the morphological changes of MI were appeared.

(b) Early ischemic changes group (n=6): This group revealed early ischemic changes which include the presence of edema, waviness of fibers, loss of cross striation, large hyperchromatic nuclei, and weak presence of neutrophils. The death in this group was happened within (12-24) hours (see photograph 1).

(c) Advanced myocardial infarction group (n=4): this group revealed the presence of macrophages, dying neutrophils, lymphocytes and plasma cells, the presence of fibroblastic proliferation in addition to collagen deposition with new blood vessels (formation of granulation tissue). All the mentioned changes indicate that the death was happened within one to two weeks.

(d) Old myocardial infarction group (n=6): This group revealed the presence of increasing deposition of collagen and decreased cellularity, some of them with dense collagenous scar, These morphological changes indicate that their age is between (2-8 weeks). [4] There are many inflammatory markers can be used as predictive markers for the risk of CHD like CRP and anticholesterol autoantibobies. Patients with acute coronary syndrome have elevation in CRP in association with their presenting symptoms. In patients with AMI the CRP levels correlate with the presence of plaque rapture.

An early study that examined CRP levels in acute coronary syndrome found that CRP identified a subset of patients with severe unstable angina at increased risk for MI and death, [17] and that agrees with our results which also showed that our lived patients had elevated levels of CRP in circulation, and in patients with acute MI it was higher than unstable angina (see figure 3).Autoantibodies to cholesterol are present in almost every healthy individual. They are natural antibodies, and their levels are independent of the amount of cholesterol in various lipoprotein fractions, and it was observed that ACHA-IgG levels were higher in CAD patients than in the age matched controls. [12]On the other hand Alving and Swartz found that sera of virtually all healthy subjects contain IgM and IgG autoantibodies against cholesterol (ACHA).[11]Amerilla et al., in 2006 found that the serum concentration of ACHA was significantly higher in patients with CAD compared with age- matched healthy controls. [17] We found that anticholesterol IgG autoantibodies were high in the patients than in the controls while IgM were higher in the controls than in the patients in addition to other results that were mentioned previously (see figures 4, 5, 6, and 7).

CONCLUSION:

This study concluded and confirmed the following findings:

1- MI affects the old people with male prevalence.
2- The potential role of inflammation in mediating pathological changes associated with IHD.
3- CRP elevated during acute coronary syndrome.
4- Anti-cholesterol auto antibodies were present in healthy individuals, but high in patients with CAD.
5- The role of ACHA in the post-mortem medico-legal diagnosis of MI.
6- The important role of medico-legal studies for different medical branches.

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