

Evaluation Of NLRP3 Inflammasome Activity In Cardiovascular Diseases Patients In Najaf Governorate

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ABSTRACT

Background: The NLRP3 inflammasome is a crucial constituent of innate immunity, mediating caspase-1 stimulation and the production of pro-inflammatory cytokines Interleukin one and Interleukin-18. Still, abnormal activating of the NLRP3 inflammasome is being connected to a number of inflammatory diseases, such as diabetes, and atherosclerotic cardiovascular disease. Cardiovascular diseases are still widespread with significant deaths and morbidity ratios. Most CVDs are caused by vascular damage, including atherosclerosis, aneurysms, and subsequent ischemic heart conditions. **Aim of the study:** Evaluation of NLRP3 inflammasome activity in cardiovascular diseases. **Methodology:** This study (cross-sectional study) included 200 individuals with cardiovascular disorders from January 2022 and May . The patients ranged in age from (1 to 80) years old, with 58 females and 142 males among the 200 cases. The serum samples of patients were obtained for screening of the presence of NLRP3 Inflammasome by ELISA using commercial kits (SUNLONG_CHINA). **Results:** Patients with cardiovascular disease were divided into 4 aging groups as, the following: The 1st age group (<20) yrs. old, The 2nd age group (20-39) yrs. old, The 3rd age group (40-59) yrs. old, and the last group, is(60-79). The results clearly showed that a high percentage of ischemic heart disease was present among all age groups compared to others types followed by structural heart disease. The current study's cardiovascular patients were higher in males compared to females by about 142(71%) vs 58(29%) of all 200 participants. The study indicates that significant results for NLRP3 inflammasome were present among age groups patients with a p-Value equal to 0.03, it also indicates that there are no significant results for NLRP3 inflammasome among sex groups of patients with a p-Value equal to 0.402. And also an association between CVD and study marker showed no significant difference between seropositive NLRP3 inflammasome with cardiovascular disease types.

Conclusions: The most common cardiovascular type in the study was ischemic heart disease. Cardiovascular disease affects men more than women, and it gets worse as people get older.

Keyword: NLRP3 Inflammasome, Cardiovascular Diseases, CVD, Najaf Governorate.

Article Information

Received: September 19, 2022; Revised: October 26, 2022; Online: December, 2022

INTRODUCTION

The inflammasome is an intracellular multimeric protein complex that combines a sensor receptor (PRR), an adaptor protein, and an effector enzyme (caspase). It catalyzes a biological reaction to defend against the imminent threat by cytokine release and death of cells. A spectrum of distinct inflammasomes can be activated by a number of damaging signals.

Moreover, depending on the kind of caspase concerned, individual inflammasomes may be split into two groups: 1) the traditional, canonical inflammasome, which activates

caspase 1 directly; and 2) the non-canonical inflammasome, which employs other caspases to transmit inflammation. (Saadatmand *et al.*, 2014). In response to microbial infection and

cellular injury, the NLRP3 inflammasome is a crucial component of the innate immune system, mediating caspase-1 activation and the production of proinflammatory cytokines Interleukin-1 and Interleukin-18. Still, abnormal activation of the NLRP3 inflammasome has been linked to a number of inflammatory diseases, such as cryopyrin-associated periodic syndromes, Alzheimer's disease, diabetes, and atherosclerosis. (Kelley et al., 2019)

In recent decades, the prevalence of metabolic illnesses ex; obesity, type 2 diabetes mellitus (T2DM), and atherosclerosis has risen, and these diseases represent great risks to human health. In the last decade, persistent inflammation has been shown to predict metabolic problems. Recent research reveals that the NLRP3 inflammasome plays a crucial role in atherosclerosis. Atherosclerosis is a persistent inflammatory condition. marked by lipid buildup, immune cell recruitment, and cytokine secretion in lesions of atherosclerosis .(Davis et al., 2011)

The NLRP3 inflammasome has also been shown to have a role in the origin and progression of cerebral and myocardial ischemic disorders, including stroke and myocardial ischemia.(Duewell et al., 2010)

Cardiovascular diseases refer to all illnesses associated with heart and circulatory system. Most common types of CVD include coronary artery diseases (CAD), cerebrovascular disease, peripheral arterial disease, and congenital heart disease.(Karunathilake & Ganegoda, 2018). Any disease of the heart and blood arteries is referred to as a CVD. Coronary artery diseases, such as angina and myocardial infarction, are examples of CVD.(Hawraa et al., 2021)

SUBJECT AND METHODS

Patients

This study (cross-sectional study) included 200 individuals with cardiovascular disorders that were clinically diagnosed by cardiology specialists. The patients ranged in age from(1 to 80) years old, with 26 females and 64 males among the 200 cases. The ". A Heart-Opening Unit " of the "Educational Hospital of Al-Sader " in Iraqi city of Najaf, registered these people as CVD patients. The study's goals and

Cardiovascular diseases are a group of disorders of the heart and blood vessels, and they include:

- Coronary artery disease (CAD) – disease of the blood vessels supplying the heart muscle such as angina, and myocardial infarction.
- Cerebrovascular disease – disease of the blood vessels supplying the brain.
- Peripheral arterial disease – disease of blood vessels supplying the arms and legs. (Anand et al., 2015)

Globally, the major causes of mortality and disability are cardiovascular illnesses, notably acute myocardial infarction (MI) and stroke. Myocardial infarction is primarily caused by the constriction of coronary arteries by atherosclerotic plaque and the instability and disintegration of the plaque. Myocardial infarction initiates an intensive and complicated inflammatory response responsible for heart healing and remodeling. (Халафли et al., 2018).

It is thought that NLRP3 inflammasome is a very probable candidate for pattern-recognition receptor in myocardial ischemia due to its ability to identify a variety of danger signals and to trigger sterile inflammation in response. According to Mezzaroma et al., inhibition of NLRP3 and Purinergic P2X7 receptors decreased inflammasome activation and cardiac cell death following coronary artery ligation in wild-type mice.(Liu et al., 2018)

NLRP3 has been identified to play a central role in the pathological progression of certain cardiovascular diseases, such as hypertension, vascular damage spanning atherosclerosis and other myocardial infarction. (Zheng et al., 2020).

objectives were explained to all groups, and they all accepted. Name, age, gender, smoking, diabetes, and blood pressure were some of the descriptive factors for all patients.

Included criteria:

Myocardial infarction, Heart failure, Congestive Heart Failure, Ischemic heart disease , Rheumatic heart disease , Angina: Stable Angina and Unstable Angina , Structural heart disease

Excluded criteria:

Congenital Cardiac problems, Cardiac arrhythmia, Valvular Heart trouble, Cardiac. Arrhythmia, Covid-19 patients, Patients who had HCV or HBV.

Approval of the Ethical Committee:

The Ethical Committee of the Kufa Medical College gave its approval to the study protocols.

Samples collection and store:

Each patient had samples of blood were taken by drawing 5 milliliters of vein blood in to the test tubes. (1 milliliter in an EDTA tube for CBC and 4 milliliters in a gel tube for biochemical analysis). The essential tests are performed on the entire blood. While blood samples in gel tubes were centrifuged at 3000 rpm for 10 Eppendorf tubes and preserved in the freezer at -20 C until the time of the immunological examination. (NLRP3 Inflammasome)

Serological technique (ELISA): The serum samples of patients were obtained for screening of the presence of NLRP3 Inflammasome by ELISA using commercial kits (SUNLONG_CHINA).

STATISTICAL ANALYSIS:

The Chi-square test and a P value of less than 0.05 were used to calculate the statistical importance of the results. It depends on SPSS 24.

RESULTS**Distribution of all patients with cardiovascular disease regarding aging groups**

Figure 1 demonstrates the distribution of CVD patients by aging group. Patient with cardiovascular disease had been divided to 4 aging groups, with the following results: The 1st age group (<20) yrs old had 27 patients(100%) from Structural heart disease. The 2nd age group (20-39) yrs old had 9 patients, with 4 patients of IHD (44.4%) and just five patients of structural heart disease(55.6%). The 3rd age group (40-59) yrs old had 100 patients, with 86 patients of IHD (86%) and just 2 patients of

Myocardopathy(2%) and 12 patients of Structural heart disease(12%). As for the last group, which is (60-79), it contained 64 cases, 57 who had ischemic heart disease (89.1%) and 4 cases had been HF (2%) with 3 cases of Myocardopathy(4.7%) as shown in (Figure 1).

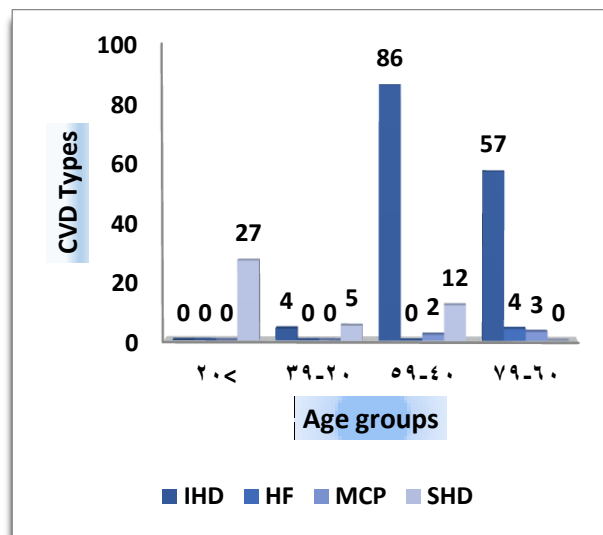


Figure 1: Distribution of all patients with cardiovascular disease regarding to ageing group.

Sex distribution of cardiovascular disease in all patients by age group

The current study's cardiovascular patients were categorized by males and females, with the total number of males being 142 cases (71%) of all 200 participants, in contrast to the overall number of female cases were 58 cases from 200 cases (29%), where male and female were divided into aging group. The dissemination of the men was the following: 18 (66.7%) cases in the group(<20) from 27 cases, while the group(20-39) had 7 (77.7%) cases from 9 and the group (40-59) had 73 cases (73%) from 100, whereas the final group(60-79) included 44 (68.8%) from 64 cases. While the distribution of the female results was as followed: 9 subjects (33.3%) for the age group (<20), 2 subjects (22.2%) for the group (20-39) and 27 subjects (27%) for the group (40-59), and the final group (60-79) was 20 subjects (31.3%) displayed by (Figure 2).

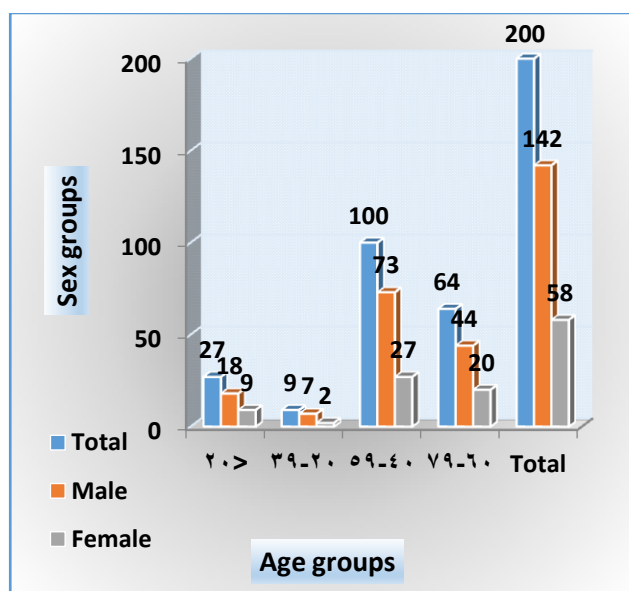


Figure (2): Sex distribution of cardiovascular disease in all patients by age group

Distribution of immunological parameters according NLRP3 inflammasome (positive,negative) in age groups .

The results in the four aging groups differ among NLRP3 inflammasome., where there was appeared in 1st group eight cases (8.7%) positive for NLRP3 inflammasome, 2nd group three cases(3.3%) positive for NLRP3 inflammasome, 3rd group forty seven(51.1%) cases positive for NLRP3 inflammasome and 4th group thirty four (36.9%) cases positive for NLRP3 inflammasome. More details displayed in the table (1). Table 1 also indicates that significant results for NLRP3 inflammasome were present among age groups patients with *p*-Value equal to 0.03.

Table (1) Distribution of immunological parameters according NLRP3 inflammasome(positive,negative) in age groups

Age groups years	NLRP3 inflammasome -ve	NLRP3 inflammasome +ve	p-value
<20	19(17.6%)	8(8.7%)	0.03
20-39	6(5.6%)	3(3.3%)	
40-59	53(49.1%)	47(51.1%)	
60-79	30(27.8%)	34(36.9%)	
total	108(100.0%)	92(100.0%)	

Distribution of NLRP3 inflammasome (positive,negative) among sex groups.

Among 92 positive cases for NLRP3 inflammasome, there was 68 cases (74%)

positive for male with 24 cases (26%) positive for female as shown in figure (3). Also, figure (3) shows that there are no significant results for NLRP3 inflammasome among sex groups of patients with a *p*-Value equal to 0.402.

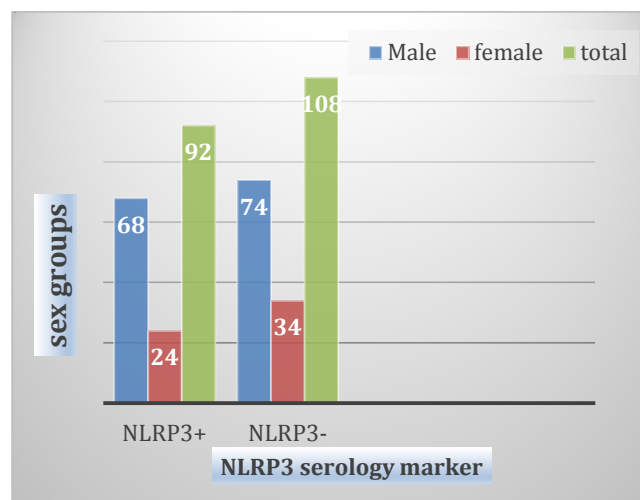


Figure (3) Distribution of NLRP3 inflammasome (+,-) among sex groups *p*-Value equal to 0.402 (not significant)

Correlations between types of cardiovascular disease and marker of research

In this study, immunological biomarker from four different types of cardiovascular disease were measured, consisting of 200 participants: 147 ISH cases, 4 HF cases, and 5 cases of cardiomyopathy with 44 cases of structural heart diseases.

The research involves an examination of the NLRP3 inflammasome, in which a total of 92 cases, having 73 cases (79.3%) of ischemic heart disease, 1 case (1.1%) of heart failure, and 18 cases (19.6%) of structural heart disease as shown in (Table 2)

Association of CVD and study marker were showed no significant difference between NLRP3 inflammasome (+ and -) with cardiovascular disease types.

Table (2):Relationship between types of cardiovascular disease and marker of research

CVD Study markers	Ischemic heart disease (IHD)	Heart failure (HF)	Myocardio-pathy (MCP)	Structural heart disease (SHD)	Total	P value
NLRP3 inflammasome +ve	73(79.3%)	1(33.3%)	0(0.0%)	18(19.6%)	92	0.101**
NLRP3 inflammasome -ve	74(68.5%)	3(2.8%)	5(4.6%)	26(24.1%)	108	
* X2 = 2.468 , df= 3 , p-Value >0.05						
** X2 = 6.221 , df= 3 , p-Value >0.05						

with *p*-Value equal to 0. 101 more details shown in table (4.14).

DISCUSSION

According to figures 1, participants having cardiovascular disease were grouped into four aged groups as seen below:

There were 27 cases of structural heart disease out of 27 cases (13%) in the first age group (<20). There were 9 patients in the second age group (20-39 years old), with 4 cases of ischemic heart disease (44.4%) and five cases of structural heart disease (55.6 %). There were 100 patients in the third age group (40-59) with 86 cases of ischemic heart disease (86%) and only 2 cases of myocardio-pathy (2 %) with 12 cases of structural heart disease (12%). The last group, consisting of those aged (60-79) years old, included 64 instances, 57 of which were IHD (89.1 %), with 4 had HF (6.3%) and only 3 cases of myocardio-pathy (4.7%). Male and female patients with cardiovascular disorders were categorized into two groups for the present research. There were 58 cases of women overall, about 29% of the total 200 cases, compared to 142 cases of men overall (about 71%) . Male and female patients were equally categorized among four age groups.

Males are distributed as follows: males had been presented by 18 cases in the category (<20) from 27 cases, representing 66.7 % of the total number of cases in this category, while the category (20-39) included 7 cases from 9 cases (77.7 %), and the group (40-59) included 73 cases from 100 cases (73%). Whereas the final

category, (60-79), included 44 (68.8%) from 64 cases, with the following distribution of females: 9 cases (33.3%), 2 cases (22.2%), and 27 (27%) and 20 (31.3%) from the total 58 female cases as shown in Figure (2).

The findings shown above are consistent with those of the American Heart Association (AHA):(Yazdanyar & Newman, 2009) in terms of the number of CVD cases among men in the second and fourth groups being more than women.

However, the above findings are in disagreement with the American Heart Association (AHA):(Yazdanyar & Newman, 2009) in terms of CVD cases among the third group in women more than in men. (Andersson & Vasan, 2018) study revealed that CVD rates are increasing in younger individuals, also this rise is probably due to an elevated prevalence of obesity, a deficit of exercise, and a poor diet. Study done by (Virani et al., 2021), demonstrated above the age of ≥ 20 years is 49.2% overall (126.9 million in 2018) and increases with age in both males and females and this agrees with my study research.

According to the (Gao et al., 2019) study, While females have a lower CVD incidence than males, they have a more severe predictor and higher fatality rates after an early cardiovascular infection. As shown in table (1), the positive results for human NLRP3 increased with increasing age, reaching a maximum in the third age group and then diminishing slightly in the fourth age group, with minimum values in the second age group among age groups. Table 1 also shows that significant results for NLRP3 were present among age groups patients with a *p*-Value equal to 0.03.

This is in good agreement with (De Biase et al., 2020) done in bovin brains that supported The theory that age-related disability in cellular housekeeping processes, as well as elevated oxidative stress, can activate the inflammatory danger sensor NLRP3. Furthermore, they demonstrated the presence of an age-related pro-inflammatory environment in ageing brains, including an upregulation of IL-1 β , elevated microglial stimulation, and further NLRP3 expression.

A study done by (Marín-Aguilar et al., 2020) in male mice that explored the molecular

mechanisms through which NLRP3 suppression could slow down cardiac ageing. Excision of the NLRP3 inflammasome shielded mice from age-related changes in insulin sensitivity, decreased levels of IGF-1 and leptin/adiponectin ratio, and reduced cardiac destruction. This was achieved by protecting the lengthening of the age-dependent PR interval, which is linked to atrial fibrillation by cardiovascular ageing and diminished telomere shortening. As shown in figure (3) that among the 92 positive cases for NLRP3, the highest percentage was in male by 68(74%) vs 24(26%) female among sex groups the same results for negative cases that in male was higher than in female groups. Also, figure (3) shows that there are no significant results for NLRP3 among sex groups of patients with a p-value equal to 0.402.

In contrast to earlier findings by (Chen et al., 2020) done in mice that suggested the loss of NLRP3 inflammasome components reduces atherosclerotic plaque size and lipid content more significantly in female mice than in male mice. OVX reduces dependent of atherogenesis on NLRP3 inflammasome components, indicating that female sex hormones make the inflammation involved in atherogenesis more sensitive.

The NLRP3 was also investigated, and 92 patients (46%) were found to be positive, with 73 cases (79.3%) of IHD, 1 case (1.1%) of HF, and 18 cases (19.6%) of SHD as shown in table (2). Study done by (Zhou et al., 2018) showed that In individuals with coronary artery disease or MI, expression of the NLRP3 inflammasome and its downstream pro-inflammatory cytokines, for example Interleukin-1 and Interleukin-18, was increased. They also noted a decrease in infarct size was seen when NLRP3 inhibitors were administered to the MI model at twenty four

hours after reperfusion immediately but not at three hours, demonstrating the short therapeutic window for pharmacological NLRP3 suppression.

A recent study done by (Tong et al., 2020) demonstrated that the NLRP3 inflammasome activation causes Expression of inflammatory genes in cardiac cells. These reactions may offer signals for macrophage recruitment, fibrosis, and myocardial malfunction in the heart. These findings suggest that preventing heart failure done by concentrating on initial inflammatory responses brought on by NLRP3 inflammasome-related signals.

Numerous studies by (Toldo et al., 2016), (He et al., 2012), (Kawaguchi et al., 2011) have proved that NLRP3 plays an important role in atherosclerosis and the development of cardiovascular illnesses.

There is no significant difference between seropositivity of NLRP3 with cardiovascular disease types with p-Value equal to 0.101. more details shown in table (2)

CONCLUSION

The most common cardiovascular type in the study was ischemic heart disease and Cardiovascular disease affects men more than women, and it gets worse as people get older.

Although there is no significant difference between seropositive NLRP3 with cardiovascular disease the development of many cardiovascular illnesses still depends on the activation of the NLRP3 inflammasome. The NLRP3 inflammasome is involved in a variety of CVDs.

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