Evaluation of Novel Biomarker of Early Atherosclerosis in Patients With Chronic Kidney Disease in Wasit Governorate

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ABSTRACT
The aim of the study was to evaluate some Biomarkers in patients with athreoscelerosis in Wasit Province. Atherosclerosis is a chronic arterial disease and a major cause of vascular death. Fatty streaks in arterial walls gradually develop into atheroma and characteristic plaques. The acute rupture of these atheromatous plaques causes local thrombosis, leading to partial or total occlusion of the affected artery. The clinical consequences of these plaques depend on their site and the degree and speed of vessel occlusion . Its major clinical manifestations include ischemic heart disease (IHD), ischemic stroke, and peripheral arterial disease. The study took place in the a hemodialysis unit Al-Zahraa Teaching Hospital in Wasit Governorate during the period from November 2023 to April 2024. This study aimed to examine risk factors (age, gender, high blood pressure, smoking, left ventricular hypertrophy, diabetes mellitus, dyslipidemia, physical inactivity) for atherosclerosis in the general population and patients with chronic kidney disease. Also studying the effect of some novel biomarkers (Homocysteine, Fibrinogen, myeloperoxidase, lipoprotein-associated phospholipase A2, pentraxin 3). The study include (90) samples: (60) patients suffering from early atherosclerosis and chronic kidney disease (dialysis stage) and 30 samples of healthy people (control group), their ages ranged from (40 to 70 years). The samples were divided into two age groups, where the first group had an average age of 40-55 years and the second group was 56-70 years old , the categories were divided equally into males and females Samples were collected based on the incidence of kidney disease first, and then the main risk factors of early atherosclerosis were relied upon, which are (smoking, age, sex, dyslipidemia, hypertension, diabetes, left ventricle valve, physical inactivity). The results of our study regarding diagnostic markers of atherosclerosis showed a significant increase in biomarkers (MPO, PTX3, HCy, FG and LpPLA2)of infected people at $p \geq 0.5$. In patients with atherosclerosis the level of new biomarkers showed a significant increase in serum of infected people compared to healthy people at $p<0.5$. This is an important indication of the importance of these biomarkers in detecting the disease.

1. Introduction

Atherosclerosis is one of the leading causes of death among the aged. The localized deposition of fat in the arteries, along with the development of smooth muscle cells and a fibrous matrix, is the primary issue with atherosclerosis. Over time, this encourages the formation of atherosclerotic plaques (1). The biological root of cardiovascular disease is atherosclerosis, and thrombosis, platelet aggregation, and unstable atherosclerotic plaque rupture will result in arterial stenosis or occlusion, resulting in acute cardiovascular illness (2). Patients with chronic kidney disease (CKD) are at an increased risk of premature mortality, mainly from cardiovascular causes (3), The association between CKD on hemodialysis and accelerated atherosclerosis was described >40 years ago. However, more recently, it has been suggested that the increase in atherosclerosis risk is actually observed in early CKD stages, remaining stable thereafter (4). Aim of the study is study some novel biomarkers (Pentraxin3, Myeloperoxidase, Lipoprotein-associated phospholipase A2, Homocysteine and Fibrinogen) and their relationship to early atherosclerosis in patient with chronic kidney disease.

2 Methodology

The present study was included a comparison of a group of (90) samples: (60) patient samples, (30) control samples. The study was conducted from the dialysis unit at Al Zahraa Teaching Hospital in November 2023 to April of 2024, and their ages ranged from (40 to 70 years) the study included 60 patients suffering from early atherosclerosis and chronic kidney disease (dialysis stage), and 30 samples of healthy people (control group). The samples were divided into two age groups, where the first group had an average age of 40-55 years and the second group was 56-70 years old , the categories were divided equally into males and females.

Samples were collected based on the incidence of kidney disease first, and then the main risk factors of early atherosclerosis were relied upon, which are (smoking, age, sex, dyslipidemia, hypertension, diabetes, left ventricle valve, physical inactivity). novel indicatorsbiomarkers of early atherosclerosis in the blood were measured (MPO, PTX3, HCy, FG and LpPLA2).

2.2 Blood Sample:

5 ml of venous blood samples were withdrawn in gel tube and clot activator for check the level of

Biomarkers	**No. of Classes of Age**		**Mean**		**F-statistic**	**P-value**	**Sig**
	Patients	Control	Patients	Control			
	40-55 and 56-70	40-55 and 56-70					
Homocysteine	60	30	643.0	535.9	5.06	0.027	Sig. *
Fibrinogen	60	30	183.9	133.6	30.01	0.000	Sig. *
MPO	60	30	183.9	133.6	10.69	0.002	Sig. *
LP-pLA2	60	30	11.9	8.5	55.20	0.000	Sig. *
PTX3	60	30	1309	914.5	52.82	0.000	Sig. *

Biomarkers

Determination of (MPO, PTX3, HCy, FG and LpPLA2) ELISA Kit: The Sandwich-ELISA principle is utilized in this ELISA kit.

Statistical Analysis: The statistical method used one way analysis of variance (ANOVA).The appropriate statistical method for testing hypotheses is F Statistics, accepting or rejecting the test hypothesis based on the probability value method associated with the F Statistics, based on the statistical significance level of 5% or 1%.

The threats

3. Result and Discussion

Table (1) shows the distribution of Biomarkers used in studying the effect of early atherosclerosis in people with chronic kidney disease, dividing them into two age groups, where the ages of the first group ranged from 40-55 years and the second age group ranged from 56-70 years.

According to the novel biomarkers of Atherosclerosis, The statistical tests for the PTX3, LP-pLA2, Fibrinogen, Homocysteine, MPO biomarkers showed a higher rate of test results in Patients groups than in the control groups and the result of the F statistical test showed that there was a significant difference for the PTX3, LP-pLA2, Fibrinogen, Homocysteine, MPO Biomarkers in Patients groups from the control groups, where the P. Value highlighted the difference because it was less than 0.05, and this indicates that there is a significant difference between the Patients groups and control groups as shown in Table (1). Table (2) shows the distribution of the results of the parameters used in the study of early atherosclerosis in people with chronic kidney disease for the age groups specific only to the group of affected people.

The statistical tests for the immunological biomarkers PTX3, LP-pLA2 showed a higher rate of test results in the first age group compared to the second age group and the result of the F statistical test showed this. P-value proved the significant difference between the two groups, as it was less than 0.05.

The average results of Homocysteine , Fibrinogen and MPO is close in proportion between the first and second categories for people with atherosclerosis and kidney failure. There is no significant

difference between the two categories because the P-value was greater than 0.05.

Table (3): Comparing the results of Novel biomarkers between the patients group and the control group according to classes of age, classes of weight, classes of height and sex.

Biomarkers	No. of Classes of Age		Mean		F-statistic	P-value	Sig
	Patients		Patients				
	40-55	56-70	40-55	56-70			
Homocysteine	30	30	644.3	629.5	0.03	0.856	Ns
Fibrinogen	30	30	185.19	168.74	1.59	0.212	Ns
MPO	30	30	111.40	109.58	0.06	0.802	Ns
LP-pLA2	30	30	11.938	10.744	10.63	0.002	Sig. *
PTX3	30	30	1317.6	1134.6	4.83	0.032	Sig. *

Biomarkers	Classes of age		Classes of weight		Classes of Height		Sex	
	F	P. value	F	P. value	F	P. value	F	P. value
Homocysteine	**1.10**	**0.339**	**0.02**	**0.984**	**1.70**	**0.144**	**3.05**	**0.084**
Fibrinogen	**3.01**	**0.009**	**1.12**	**0.330**	**2.18**	**0.064**	**0.97**	**0.326**
MPO	**10.69**	**0.002**	**1.80**	**0.172**	**0.73**	**0.606**	**0.40**	**0.529**
LP-pLA2	**55.20**	**0.000**	**0.91**	**0.408**	**2.32**	**0.070**	**0.08**	**0.773**
PTX3	**52.82**	**0.000**	**0.09**	**0.914**	**0.54**	**0.743**	**0.01**	**0.993**

The result of F test related to the effect of the factor (classes of Age), it showed significant statistical significance for each of the parameters (PTX3, LP-pLA2, Fibrinogen, MPO), because the (P.value) accompanying the F statistic for each of the mentioned parameters It was less than the level of (0.05), and this indicates that the levels of measuring parameters were greater for infected persons compared to non-infected persons. while the Homocysteine parameter showed no significance because the (P.value) accompanying the F statistic for each of the parameter was greater than the level (0.05) as illustrated in table (2) . This indicates that the (classes of Age) factor has no significant effect on the

measurement levels of the aforementioned parameter. The F test result related to the effect of the factor (classes of weight), it did not show any significance for each of the parameters (PTX3, LP-pLA2, Fibrinogen, Homocysteine, MPO), because the (P.value) accompanying the F statistic for each of the mentioned parameters was greater than the level (0.05). This indicates that the (Diabetes) factor has no significant effect on the measurement levels of the aforementioned parameters as illustrated in table (2) .

Depending on the F test result related to the effect of the factor (classes of Height), it did not show any significance for each of the parameters (PTX3, LP-pLA2, Fibrinogen, Homocysteine, MPO), because the (P.value) accompanying the F statistic for each of the mentioned parameters was greater than the level (0.05). Depending on the F test result related to the effect of the factor (Sex), it did not show any significance for each of the parameters (PTX3, LP-pLA2, Fibrinogen, Homocysteine, MPO), because the (P.value) accompanying the F statistic for each of the mentioned parameters was greater than the level (0.05) as illustrated in table (3).

Homocysteine

The significant increase recorded in the results of this study regarding the Homocysteine amino acid, Many studies relate to elevated Hcy role in the development of various forms of vascular diseases (5).Three emerging risk factors potentially useful in predicting future cardiac events are electron-beam computed tomography (EBT), homocysteine(HCY), and C-reactive Protein (CRP) (6). Hcy may cause CVD through various mechanisms such as the increased proliferation of muscle cells that cause narrowing of vessels, alter blood coagulant properties, cause oxidant injury to the vascular endothelium, and damage arterial walls (7).

The results of the Homocysteine concentration were consistent with what the researchers indicated (5)(8)(9).

The results of the study showed a significant correlation between smoking and homocysteine measurement between the infected group Compared with the control group, This result is proven because Cigarette smoking is known to be associated with a raised plasma homocysteine level. Smokers also tend to have lower levels of the B-vitamins, folate, vitamin B6 and vitamin B12, all of which affect homocysteine levels by acting as co-factors (vitamins B6 and B12) or co-substrate (folate) for the enzymes controlling homocysteine metabolism (10).

There was No association between fibrinogen levels and smoking effect in this study.The possibility is due to the reasons mentioned in the study of (11), response to 2 weeks cessation in smoking, the rates of fibrinogen synthesis were reduced to levels comparable with those of the non-smokers The results from both studies support the proposal that smoking induces fibrinogen synthesis, and this effect can be reduced by abstention from smoking (11).

another studies was found that there was a significant relation between smoking effect to fibrinogen level, fibrinogen values have been found to be higher in smokers than in nonsmokers (12).

As for the study on (effect the smoking factor on myeloperoxidase biomarker), the results of this study showed a significant correlation, MPO emerges as a critical contributor for the development and progression of coronary artery disease in smokers, and may evolve as a novel biomarker in patients with cardiovascular disease(13).

Fibrinogen

As for the study on the Fibrinogen protein, a significant increase in the concentrations of this protien was recorded in patients suffering from Atherosclerosis and CKD.

The results of numerous epidemiological studies indicate that increased plasma fibrinogen concentrations are a risk factor for ASCVD (14). elevated plasma fibrinogen concentration was related to carotid IMT, a surrogate marker of atherosclerosis, Elevated levels of fibrinogen are

strongly associated with human vascular disease, fibrinogen plays a causal role in vascular disease progression. Elevated plasma fibrinogen may promote vascular disease by increasing blood viscosity, promoting fibrin formation, enhancing platelet-platelet interactions, or by other, suggesting that fibrinogen may in part mediate the downstream effects of risk factors on atherosclerosis (15).

These results of the concentration Fibrinogen were consistent with what the researchers indicated (16) as this study showed an increase in the concentration of fibrinogen, Another study also showed that fibrinogen is a more sensitive indicator of early atherosclerosis (15)(17).

myeloperoxidase

The significant increase recorded in the results of this study regarding the myeloperoxidase enzyme may be attributed to the Myeloperoxidase has been implicated as a participant in atherosclerosis through mechanisms related to its role in inflammation (18).

Recent studies have begun to unravel some of the specific oxidation pathways that contribute to lipoprotein and lipid oxidation during vascular disease. One pathway for which significant data are now available involves the leukocyte protein myeloperoxidase (MPO). MPO is present in human atherosclerotic lesions (18), a considerable number of epidemiological and clinical studies have demonstrated an association between increased concentrations of MPO and CVD (19).

The results of the myeloperoxidase concentration were consistent with what the researchers indicated (18)(19) in contrast was not consistent (20) suggested the role of MPO in cardiovascular disease remains unclear. Indeed, it is interesting to note how little is really known about(20).

LP-pLA2

Diseased arteries contain lipoprotein-associated phospholipase A2 (Lp-PLA2), which is Mostly made by mast cells, T lymphocytes, and monocytes (21).

As for the study on (lipoprotein-associated phospholipase A2), the results of this study showed a significant increase in the concentrations of (lipoprotein-associated phospholipase A2) in infected people ,The Epidemiological studies have suggested that elevated circulating lp-PLA2 is predictive of increased cardiovascular risk (22)(23).

PTX3

Recently, pentraxin 3 (PTX3) has also been implicated in cardiovascular events. PTX3, a prototypical member of the long pentraxin family, has a C-terminal sequence homology with the classic short pentraxins, CRP and serum amyloid P component. PTX3 is abundantly produced by various cells in atherosclerotic lesions, including monocytes, macrophages, endothelial cells, vascular smooth muscle cells, fibroblasts, dendritic cells, and adipocytes (24). More recently, PTX3 itself has been recognized as critical determinant of the endothelial dysfunction. The overexpression of PTX3 blunts nitric oxide production through the up-regulation of matrix metalloproteinase-1 and P-selectin (25).

Conclusion

This study is consistent with previous studies referred to by researchers (26)(27). Suggest that PTX3 levels reflect local inflammation at atherosclerotic lesions.

As for the results of the study on risk factors (Hypertension, diabetes, height category, weight category, and gender), they are not significant. In the results of these factors were recorded. This may explain the lack of significance due to the difference between age groups, gender, and the large number of pathogens (28), the results of the study are not consistent with previous studies (29)

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