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Study of the Midkine level and its Correlation with toxic and Essential Metals in Patients with Atherosclerosis

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Abstract

The research includes studying of level for midkine (MK), toxic metals (TMs) (Including: Arsenic(As), Mercury(Hg), Cadmium(Cd)) and essential metals (EMs) (Including: Magnesium(Mg), Calcium (Ca), Iron(Fe)) in serum for atherosclerosis patients (APs). The study was done in Mosul city on (107) samples, including 57 for APs and 50 who were healthy as a control group.

The results showed a significant increase in MK, As, Hg and Cd levels for all studied (All, males and females) groups compared with control groups. While, the results of measurements of EMs indicated that there was a significant decrease in Ca, Mg and Fe compared with control groups and this indicates the effect of atherosclerosis.

The study indicated that the MK increased in patients with atherosclerosis, and it increased in males more than in female patients, in addition, to increase in TMs measured (As, Hg and Cd) helped increase the pathogenesis of atherosclerosis by increasing the level of MK and decreasing levels of Ca, Mg and Fe. Thus, increasing the intake of nutritional supplements from essential minerals is important in reducing the severity of infection. And that MK can be considered a good indicator of the occurrence of atherosclerosis.

Keywords: Atherosclerosis, Midkine, Arsenic, Mercury, Cadmium, Essential metals.

Introduction

Atherosclerosis is a persistent multifocal immuno-inflammatory sickness of the arteries. The atherosclerotic method starts offevolved in early teenage years and keeps at some point of life. It is characterised via way of means of accumulation of cholesterol, infiltration of macrophages, proliferation of clean muscle cells and accumulation of connective tissue additives withinside the intima of the vessel wall [1]. Accumulation of lipids and SMC and monocyte derived macrophages result in the formation of a fibrous plaque. Although a fibrous plague can develop sufficiently massive to dam blood flow, the maximum crucial medical hardship is an acute occlusion because of the formation of a thrombus or blood clot, ensuing in myocardial infarction or stroke[2].

Midkine (MK) its foremost characteristic changed into believed to orchestrate embryonic development[3]. Maximum of the research on MK addressed its position in malignant sicknesses and recommended a destructive impact for the host [4]. A extensive a part of the literature on MK has proven its cappotential to sell inflammatory responses [5]. In addition, latest research confirmed that MK serves as a primary regulator of angiogenesis and arteriogenesis throughout pathological situations withinside the vascular system. In mild of the fast-developing burden of cardiovascular sicknesses, which might be predominantly related to atherosclerosis for example with inside the heart, interventional techniques to modulate arteriogenesis, the boom of pre-current arteriolar connections bypassing an occluded artery, can also additionally come to be novel noninvasive remedy modalities for sufferers with ischemia-associated pathologies [6].

A look at carried out in Cd exposure extended overall cholesterol ensuing in atherosclerotic plaque

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formation within the aorta [7]. In a few studies, with a excessive stage of As in groundwater, an affiliation became discovered among As may be effects on formation of atherosclerosis and produced other heart diseases[8]. Beside of, Hg toxicity is certainly strongly related to hypertension, CHD, MI, carotid artery occlusion, and atherosclerosis[9].

Ca, Mg and Fe levels play one-of-a-kind roles that have an effect on the method of atherosclerosis. For example, it changed into discovered that Fe deficiency can impair cardiomyocyte mitochondrial feature and electricity supplement, main to cardiac dysfunction [10, 11]. An extra quantity of Fe also can be poisonous through generating hydroxyl radicals through the Haber–Weiss–Fenton reactions, inflicting oxidative harm to mobile additives like lipids, proteins, and DNA[12].

This study aims to estimate the concentrations of MK, poisonous and EMs in sufferers with atherosclerosis in comparison to wholesome controls, in an effort to check the quantity of this impact at the pathological condition, in addition to understanding the position of MK in atherosclerosis sufferers and its relationship to TMs which includes cadmium, arsenic, mercury and may be used as biochemical markers to decide the severity of atherosclerosis in sufferers.

Materials and Methods:

Samples were collected during the period from January 2021 to July 2021 from patients in the Cardiac Surgery Center and inpatients at Ibn Sina Teaching Hospital / Mosul and Mosul General Hospital / Mosul. The clinical diagnosis in each case is made according to a cardiovascular specialist. This study included (57) patients are between the ages of (26-86) years. At the same time, samples of the healthy group were collected, with (50) samples with negative results for cardiovascular diseases, or chronic diseases, and they are age-matched to the patients, and they have no history of any disease.

10 mL of blood and allow to clot, serum was separated by using speed at (4000 × g) for ten mints, divided into (3) sections and kept frozen at (-20 °C) until analysis. Tables (1, 2 and 3) showed a description of healthy and APs groups (All, Males and Females groups), which indicated to non-significant for age and body mass index (BMI) for all groups, males and females.

Table 1: Description of healthy and all APs groups.

Parameters	Healthy group n=50		Patients group n= 57		P value
	Mean	SE	Mean	SE	
Age (Year)	54.54	1.85	56.75	1.78	0.12
Weight(Kg)	80.82	2.33	79.42	1.43	0.813
Height(cm)	171.22	1.16	169.8	1.56	0.495
BMI(kg/m^2)	27.56	0.74	27.46	0.52	0.869

Table 2: Description of males for healthy and APs groups.

Parameters	Healthy group n=25		Patients group n= 35		P value
	Mean	SE	Mean	SE	
Age (Year)	53.68	2.1	54.57	2.14	0.12
Weight(Kg)	84.16	2.85	80.34	2.14	0.583
Height(cm)	176.1	1.34	170.6	2.37	0.096
BMI(kg/m^2)	27.16	0.85	27.38	0.75	0.514

Table 3: Description of females for healthy and APs groups.

Parameters	Healthy group n=25		Patients group n= 22		P value
	Mean	SE	Mean	SE	
Age (Year)	57.4	2.89	60.23	3.03	0.18
Weight(Kg)	77.48	3.63	77.95	1.47	0.949
Height(cm)	166.4	1.33	168.4	1.49	0.358
BMI(kg/m^2)	27.96	1.24	27.6	0.645	0.750

Midkine activity has been determined by using kit type Bioassay technology Laboratory, Cat. No E1633Hu)[13].

Ca, Mg and Fe levels were estimated using the colorimetric methods and used standard containers from the French company BIOLABO, For Ca determination which depend on the reaction of o-Cresolphthalein with Ca ion and the formation of a complex with a red color, as its intensity is measured at 570 [14]. Magnesium estimated by xylidyl was used as a blue complex that is a colored complex and the intensity of the color of this complex corresponds to the concentration of magnesium in the sample [15]. The iron concentration was estimated using separation of the ferric ion from the transferrin in the acidic medium and the iron ion is reduced to the ferrous ion, then the ferrous ion is formed in a complex with ferine to give a colored complex proportional to the intensity of its absorption with the iron concentration the sample[16].

In addition, the concentration of arsenic, mercury and cadmium in the serum was estimated using the atomic absorption spectrophotometer, by taking 1 mL of the serum and placing in clean volumetric bottles, 1 ml of concentrated nitric acid was added to it, then the nozzles were closed and left for two hours, then it was heated at a temperature of 60°C for a period of (3) hours, then it was left to cool, then a centrifugation process was carried out to obtain the clear liquid, and the samples were diluted with ion-free distilled water to reach the volume to (15) mL, and the atomic absorption of these samples was measured and the wavelength of each mineral was determined and the atomic absorption measurement was converted into concentration units depending on the prepared standard curves at different concentrations of these minerals used in the study[17].

SPSS-26 software was used for statistical analysis and p-value threshold of *Significant at ($p \leq 0.05$),**Significant at ($p \leq 0.001$),***Significant at ($p \leq 0.0001$) was used to indicate statistical significance.

Results and Discussion:

1: Levels of MK of all, male and female APs groups:

The results shown in Tables (4, 5 and 6) indicate that there is increase in MK for APs at $p \leq 0.0001$ for all patients, male and female groups, and these results are showed in Zhang *et al*. (2021) that there is an increase in the level of MK in the serum with Atherosclerosis [18].

MK has attracted increasingly interest because it is involved in a number of physiological and pathological methods and has been proven to irritate atherosclerosis thru a couple of mechanisms. Although MK can promote macrophage lipid accumulation and macrophage polarization is a key of infection and is carefully related to atherosclerosis progression[19, 20], however little or no is thought approximately its function. The inclined plaques show skinny fibrous caps and massive necrotic middle areas with at risk of rupture[21].

Table 4: MK and some TMs in all APs group .

Biochemical Parameters#	Healthy group		Patients group		P value
	Mean	SE	Mean	SE	
MK	159.7	6.1	348.2	20.86	0.0001***
As	0.049	0.004	0.073	0.005	0.028*
Hg	0.058	0.003	0.11	0.024	0.042*
Cd	0.012	0.007	0.065	0.009	0.0001***

Table 5: MK and some TMs in male APs group.

Biochemical Parameters#	Healthy group		Patients group		P value
	Mean	SE	Mean	SE	
MK	152.3	5.91	383.4	29.8	0.0001***
As	0.048	0.007	0.083	0.008	0.036*
Hg	0.059	0.004	0.177	0.014	0.022*
Cd	0.014	0.008	0.067	0.012	0.0001*

Table 6: MK and some TMs in female APs group .

Biochemical Parameters#	Healthy group		Patients group		P value
	Mean	SE	Mean	SE	
MK	167.2	10.81	292.3	21.84	0.0001***
As	0.052	0.007	0.093	0.027	0.016*
Hg	0.057	0.003	0.138	0.025	0.041*
Cd	0.01	0.001	0.063	0.015	0.0001*

*Significant at ($P \leq 0.05$) , *** Significant at ($P \leq 0.0001$).

#MK expressed in pg/ml; As, Hg and Cd expressed in µg/dl.

2: Level of TMs in APs in all, male and female groups:

2.1: Arsenic (As):

The results shown in Tables (4, 5 and 6) indicate that there is high in As for atherosclerosis at $p \leq 0.05$ significant at for all, male and female groups, and these results same the Velmurugan *et al*. (2020)[22].

As-triggered hepatic harm effects in impairment of hepatic detoxing mechanisms which could cause higher accumulation of organophosphate residues[23]. There also are opportunities that interplay of As and organophosphates produce a brand new As species which could have greater destructive impact at the machine main to metabolic and vascular dysregulation. The heavy use of artificial phosphate fertilizers results in extension of As trouble to the one of a kind areas of the arena that aren't formerly diagnosed as herbal As wealthy areas[24].

2.2: Mercury (Hg):

The results shown in Tables (4, 5 and 6) indicate that there is a significant increase in the levels of Hg in APs at $p \leq 0.05$ for all, male and female patients groups, and the same of previous results[25].

Hg and its compounds set off poisonous damages withinside the body thru numerous mechanisms. Researchers have proven that the poisonous mechanisms of all styles of Hg have high similarities[26]. Hg binding with sulfhydryl may be caused changed DNA, RNA, and ribosomal proteins[27], and decreased glutathione (GSH) and lipoeic acid [28] .

Hg-induced OS caused either by increasing the production of oxidative agents or by inducing a decrease in antioxidant activity[29]. The increase of OS induces, via various pathways, endothelial inflammation first and then endothelial dysfunction[30] followed by the development of atherosclerosis, thrombophilia diathesis, and risk of ischemic phenomena through vessel obstruction or vasospastic events [31].

2.3: Cadmium (Cd)

The results shown in Tables (4, 5 and 6) indicate that there is a high in Cd for APs at $p \leq 0.0001$ for all, male and female groups, and these results same as Barregard *et al*. (2021)[33].

Cd from diet or smoking as the main sources. Cd accumulates mainly in the kidneys and can risk factor for cardiovascular disease[34, 35], and development and progression of atherosclerosis [35].

There are experimental research and populace research, suggesting that Cd exposure is related to a proatherosclerotic lipid profile[34]. A key step in plaque formation is the trapping of LDL-containing lipoprotein particles through proteoglycans with inside the arterial wall. It has been proven in research that Cd promotes of atherogenic lipoproteins [34].

3: Levels of EMs in APs for all, male and female groups:

3.1: Calcium (Ca)

The results shown in Tables (7 and 8) indicate that there is lower for Ca in APs at $p \leq 0.05$ for all and male patients and significant decrease at $p \leq 0.001$ for female groups, and these results are same with Kalampogias *et al*. (2016)[36].

In the beginning, atherosclerosis is a continual inflammatory circumstance that starts offevolved with the formation of calcified plaque, influenced by a number of of various factors in the vascular wall in huge and mid-sized arteries.Ca mineralization of the lumen withinside the atherosclerotic artery promotes and solidifies plaque formation inflicting narrowing of the vessel. Arterial calcification emerges from special types, the medial-elastin established and the intimal, which can be associated with atherosclerosis without delay because of osteoblast differentiation of vascular SMC. The deposition of minerals withinside the shape of calcium (Ca^{2+}) to start with emerges from octacalcium phosphate [$Ca_8H_2(PO_4)_6.5H_2O$] to the shape of hydroxylapatite [$Ca_{10}(PO_4)_6(OH)_2$] [36]. Artery calcification happening in atherosclerosis is attached with a high risk of cardiovascular events[37].

Table 7: Ca, Mg and Fe in all APs group as compared with Healthy group.

Biochemical Parameters	Healthy group n=50		Patients group n= 57		P value
	Mean	SE	Mean	SE	
Ca (mg/dl)	10.41	1.6	8.18	0.066	0.041*
Mg(mg/dl)	2.53	0.44	1.03	0.043	0.001**
Fe(µg/dl)	13.8	0.02	4.3	0.005	0.0001***

Table 8: Ca, Mg and Fe in male APs group as compared with Healthy group.

Biochemical Parameters	Healthy group n=25		Patients group n= 35		P value
	Mean	SE	Mean	SE	
Ca (mg/dl)	12.12	0.20	8.21	0.088	0.024*
Mg(mg/dl)	2.168	0.067	0.977	0.059	0.0001***
Fe(µg/dl)	10.3	0.023	4.0	0.001	0.001**

Table 9: Ca, Mg and Fe in female APs group as compared with Healthy group.

Biochemical Parameters	Healthy group n=25		Patients group n= 22		P value
	Mean	SE	Mean	SE	
Ca (mg/dl)	9.70	0.067	7.31	0.101	0.001**
Mg(mg/dl)	2.89	0.088	1.11	0.057	0.039*
Fe(µg/dl)	17.3	0.033	4.9	0.013	0.0001*

*Significant at (P≤0.05) **,** ** Significant at (P≤0.001)**,** *** Significant at (P≤0.0001)**.**

3.2: Magnesium (Mg)

The results shown in Tables (7, 8 and 9) indicate that there is a significant lower in the Mg for APs at $p \leq 0.001$ for all patients and at $p \leq 0.0001$ in male patients group and at $p \leq 0.05$ in female patients group, these results same with Kostov *et al*. (2018)[38]. Mg lack can lead to increased of triglycerides and cholesterol may be cause atherosclerosis [39]. When the Mg decrease increased the OS may be increased the many disease for example the APs by changes in lipid metabolism, which enhance atherosclerotic and that associated with arterial hypertension, Eating a wholesome weight loss plan that gives the encouraged quantity of Mg may be the ideal approach for supporting manipulate blood pressure [38].

3.3: Iron (Fe)

The results shown in Tables (7 and 9) indicate that there is a significant lower at $p \leq 0.0001$ for Mg in APs for all and female groups, and significant decrease at $p \leq 0.001$ (Table (8)) in male patients group and these results are consistent with Meng *et al*., (2022)[40].

Fe is essential for many proteins and enzymes and keeps mitochondrial function, DNA synthesis and repair, and cell growth and death[41, 42]. Fe ions have an effect on the occurrence of atherosclerosis and neurological diseases with hydrogen peroxide can oxidize numerous materials for example oxidizing LDLs and development of cardiovascular disease by damage vascular endothelial cells[43, 44].

Conclusion

The study indicated that the MK increased in patients with atherosclerosis, and it's increased in males more than in female patients, in addition, the increase in TMs measured (As, Hg and Cd) helped increase the pathogenesis of atherosclerosis by increasing the level of MK and decreasing levels of Ca, Mg and Fe. Thus, increasing the intake of nutritional supplements from essential minerals is important in reducing the severity of infection. And that MK can be considered a good indicator of the occurrences of atherosclerosis.

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