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ORIGINAL ARTICLE

A CLINICAL STUDY OF LIPOCALIN 2 AND ITS RELATION WITH OXIDATIVE AND ANTIOXIDATIVE FACTORS IN ARTHRITIS

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Summary

Background: Lipocalin-2 (LCN2) is a protein that has been associated with skeletal muscle regeneration, but details regarding its role in Arthritis remain unclear.

The aim of the current study was to investigate LCN2 levels of Arthritis patients and its relationship with oxidative and antioxidative factors

Methods: The study includes (125) blood samples of persons aged 20–65 years were divided into a control group (apparently healthy) consisting of 55 samples [31female, 24 males] and a Patient group consisting of 70 samples [37female, 33 males] who were attending the bone diseases consultation unit at the Ibn Sina Teaching Hospital in Mosul, Iraq.

Venous blood samples (10 ml) were collected after overnight fasting. To conduct Clinical analyses: Serum LCN2 level was determined by ELISA, also Malonaldehyde, glutathione, vitamin E, vitamin C, peroxy nitrite, peroxidase, and aryl esterase were estimated

Results: The findings revealed a significant increase in the levels of LCN2 in Arthritis compared to the control group and there was a significant decrease in the concentration of vitamin C, glutathione, vitamin E and the activity of the arylesterase in serum of patients with arthritis compared with the control group. Also, a significant increase in the activity of peroxidase, concentration of peroxynitrite and malondialdehyde for patients than a control group

Conclusion: These findings imply that LCN2 may play a substantial role in iron-related oxidative stress damage in arthritis. Thus a therapeutic candidate target for treatment.

Key words: Lipocalin-2; oxidative stress; Arthritis; BMI

Introduction

Neutrophil granules were the original source of the 25 kD glycoprotein known as neutrophil gelatinase-associated lipocalin (NGAL, lipocalin 2, Lcn2) (1). Adipokine lipocalin-2 (LCN2) is secreted and carries iron, lipids, and tiny hydrophobic compounds and its thought to play a role in maintaining iron homeostasis (2). Although it is believed that the primary function of lipocalins is to transport small ligands, they have also been connected to a variety of other functions, such as retinol transport, cell homeostatic mediation, prostaglandin synthesis, and immune response regulation (3).

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The uncontrolled iron buildup is a key source of reactive oxygen species (ROS), as iron is a transition metal that can switch between different oxidation states depending on physiologic conditions (4). Therefore, modifications in the iron homeostasis of the muscle might result in muscle deterioration and reduced muscular function and an increased iron concentration and oxidative damage are related to muscle atrophy (5-6). Despite the fact that abnormal oxidative stress can cause muscular atrophy due to iron excess. Under stressful circumstances, Lcn2 expression increases in a range of illnesses like cancers, infection, inflammation, and alcoholism, where the production of free radicals has been linked to various ailments (4, 7).

Arthritis is a common term used to describe a group of chronic inflammatory disorders of the joints (8), which is a widespread disease that is not defined as a single disease, but as a group of diseases that affect the joints and mainly target the synovial membrane, cartilage and bone. It is described as chronic or acute inflammation of the joints, often causing structural damage and pain (9). Arthritis can be a chronic condition or a transient effect of bacterial or viral infection (10). Arthritis is characterized by an imbalance between the production and inactivation of reactive oxygen species (ROS) causing increased oxidative stress (11-12). Several oxidative stress mechanisms have been proposed including chronic inflammation, tissue dysfunction, and ROS formation, have been proposed to increase oxidative stress (13-14).

The current study's objective was to clarify LCN2's function in arthritis and its connection to oxidative stress.

Materials and methods

Ethical approval

This study has received ethical approval from the Iraqi Ministry of Health - Nineveh Health. Before collecting samples, consent was acquired from each participant.

Study design

This research was a case-control study for the control and patient group:

- **The Patients group:** (70) samples with arthritis which included (37) females and (33) males ages (20–65 years), all arthritis patients visiting the Ibn Sina Teaching Hospital in Mosul, noting that the patients were diagnosed by specialized doctors. Patients' information was recorded according to the questionnaire paper.

- **The control group:** (55) samples of healthy individuals which included (31) females and (24) males who matched the patient's age and did not have diabetes, or any other medical conditions, as well as no use of any medication.

Measurement demographic and biochemical parameters:

- Blood pressure: Their blood pressure was checked using an automated device and taken twice (15).
- Body mass index (BMI): Weight in kg/height in m2 was used to determine (BMI) (16).
- prepare of serum: (5 ml) of venous blood was drawn after an overnight fast [of 12 hours] from all participants, and blood was centrifuged for 10-15 minutes at 3500 (rpm) to get the serum.
- lipocalin-2: the was measured by using an Enzyme-Linked Immunosorbent Assay (ELISA) kit from SUN LONG Biological Technology Co., Ltd kit (China).

Estimation of the activity of aryleasterase, peroxidase, the concentration of malondialdehyde (MDA), glutathione (GSH) (17), vitamin C, vitamin E, (18) and peroxynitrite (19) in the serum.

Data Analysis: The data is shown as mean ± SE. The comparison between the arthritis group and the control group using the t-test. Pearson correlation coefficient (r) was applied to determine the relation between parameters based on linear regression analysis. P values ≤ 0.05 are considered significant.

Results

Baseline Anthropological Characteristics of the Study Participants

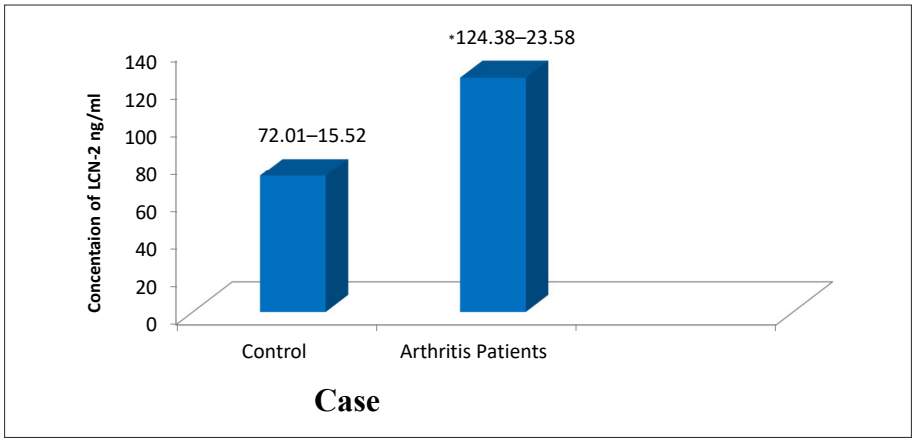
Table 1 provides the Anthropological features of arthritis and control groups. When comparing the two groups, the participants had anthropometric details showing the age, sex, BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) of patients were higher significant at level ($P \leq 0.01$) than those of the controls.

Variables	Control (Mean ± SD)	Patient Mean ± SD
No. of subjects	55	70*
No. of subjects F/ M	31/24	37/ 33*
Age (years)	39.24 ± 20.70	48.5 ± 10.23*
Age of Females (years)	36 ± 15.2	49.56 ± 7.3*
Age of Males (years)	41.25 ± 16.7	47.06 ± 8.78*
BMI (kg/m^2)	29 ± 9.5	30.69 ± 8.78*
BMI of Females (kg/m^2)	28.89 ± 10.7	31.9 ± 7.37*
BMI of Males (kg/m^2)	30.57 ± 5.20	30.3 ± 8.38*
SBP / DBP (mm Hg)	13.2 ± 1.2 / 8.1 ± 0.5	14.6 ± 1.5*/ 9.3 ± 0.8*

* Significant at the level ($P \leq 0.01$)

Lipocalin-2 for Arthritis

The concentration of lipocalin-2 (LCN-2) in the serum of patients with arthritis was estimated, and the results showed in Figure (1) a significant increase in the concentration of LCN-2 in the serum of patients with arthritis at the level ($P \leq 0.001$) compared to its concentration in the serum of the control group.



* Significant at the level ($P \leq 0.001$)

Figure 1. Lipocalin-2 concentration in the control and patients groups.

Oxidative Stress Factors for Arthritis

There was a significant decrease at the level ($P \leq 0.01$) in concentration of vitamin C, glutathione, vitamin E and the activity of the arylesterase in serum of patients with arthritis compared with the control group, as the results showed in Table 2. Also, a significant increase at the level ($P \leq 0.01$) in the activity of peroxidase, concentration of peroxynitrite and malondialdehyde for patients than control group.

Table 2. Oxidative Stress Factors for Arthritis and Control Groups.

Oxidative Stress Factors	Control (Mean ± SD)	Patient Mean ± SD
vitamin C (µmol/l)	26.2 ± 6.31	11.18 ± 3.20*
Glutathione (µmol/l)	4.98 ± 1.59	1.59 ± 0.53**
vitamin E (µmol/l)	34.22 ± 4.87	18.02 ± 2.82*
Aryleasterase (U/ml)	116.52 ± 4.11	88.89 ± 5.4*
Peroxidase (U/ml)	88.92 ± 21.4	130.04 ± 28*
peroxynitrite (µmol/l)	62.11 ± 6.28	91.22 ± 12.17*
Malondialdehyde (µ mol/l)	1.26 ± 0.31	3.96 ± 0.93**

* significant at the level ($P \leq 0.01$)
** significant at the level ($P \leq 0.001$)

The Relationship between Lipocalin-2 and Oxidative Stress Factors for Arthritis Group

The results in Table 3 showed that there was a significant positive correlation at the level ($P \leq 0.05$) between lipocalin-2 with glutathione. There was also a significant inverse correlation between lipocalin-2 with the activity of peroxidase and the concentration of peroxynitrite in the arthritis group.

Table 3. The Relationship between Lipocalin-2 and Oxidative Stress Factors for Arthritis Group.

Lipocalin-2	
Oxidative Stress Factors	**R-value**
vitamin C	0.433
Glutathione	+ 0.52*
vitamin E	0.58*
Aryleasterase	0.383
Peroxidase	-0.72*
peroxynitrite	-0.65*
Malondialdehyde	0.48

*Significant at the level ($P \leq 0.05$)

Discussion

The results of the study showed that the highest incidence of arthritis was in middle age, and for women was higher than for males, as well as for people with a higher body mass index. In the years immediately following the cessation of menstruation, this puts women at greater risk of early menstruation (20-21). Overweight people also have a higher risk of developing arthritis. Excess weight puts more pressure on joints, especially weight-bearing joints such as the hips and knees (22-23).

The results showed high blood pressure in people with arthritis, and this is consistent with (23-24) that most arthritis patients suffer from high blood pressure, and this is associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs), which are commonly used. used to treat this common disorder by increasing blood pressure and disrupting blood pressure treatment.

A high concentration of lipocalin-2 was found in the blood serum of patients with arthritis, and this is consistent with (26), the reason is that lipocalin-2 is secreted in response to inflammation and stimulates its secretion by pro-inflammatory cytokines, and its concentration is associated with many markers of inflammation.

An imbalance between the generation of oxidative substances like reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the body's natural antioxidant defenses is referred to as oxidative stress (13, 18).

A low concentration of glutathione was found in patients with arthritis. This is due to the role of glutathione in reducing oxidative damage, as it is a non-enzymatic antioxidant that removes free radicals and reduces oxidized LDL (27, 28), Also, a decrease was found in the concentration of the vitamin E in arthritis patients, as vitamin E is a non-enzymatic antioxidant that suppresses free radicals and prevents the oxidation of polyunsaturated fatty acids, and thus works to protect joint tissues from high oxidative stress due to inflammation. Thus, its consumption increases (29, 30).

The concentration of vitamin C in the serum of patients with arthritis also decreases, and the reason is due to the fact that vitamin C is one of antioxidants whose consumption increases in the process of reducing oxidative stress, and it was found associated between the low concentration of vitamin C in the serum and the risk of muscle degeneration and inflammation (30, 31), a decrease was found in arylesterase in arthritis patients, as the aryl esterase binds to HDL molecules to exercise its function as an antioxidant (32-33).

Arthritis is caused to high levels of free radicals and oxidative stress processes, which leads to an increase in lipid peroxides, and thus an increase in the concentration of malondialdehyde is due to the increased production of free radicals from the oxidation of unsaturated fats in joint tissues and adjacent tissues as well (34-35). The high activity of peroxidase is due to the role of oxidative stress in Arthritis, as peroxidase enzymes are released from activated immune cells at sites of inflammation to provide a defense mechanism against bacteria and pathogenic microorganisms, and works to protect tissues from damage by scavenging free radicals (18-36).

Also, In the case of inflammation, the number of free radicals increases, including (NO.) due to oxidative stress, which leads to an increase in the production of peroxynitrites (ONOO.) and thus leads to deterioration of the joints as a result of a higher rate of inflammation (14-37).

Arthritis Group showed a direct correlation between Lipocalin-2 and antioxidant factors (glutathione). Also, an inverse correlation was found between Lipocalin-2 and oxidant factors (peroxidase and peroxynitrite) may be due to the consumption of antioxidants and the increase of free radicals as a result of the increase in oxidative stress processes. Perhaps this is due to the role of lipocalin as an antioxidant by increasing the levels of glutathione reductase and inhibiting the activity of iron-related enzymes because it has the ability to capture or transport iron, including peroxidase enzymes (4-7).

Lipocalin 2 (LCN2), a multifunctional protein, acts as an iron transporter and antioxidant. Lipocalin 2 (LCN2) appears to inhibit the activity of iron-binding enzymes because it has the ability to scavenge iron and thus contribute to both oxidative stress and inflammation (3-6).

Conclusion

Arthritis is caused by the increased production of free radicals from the oxidation in joint tissues and their adjacent which raises levels of reactive oxygen species (ROS) in the joint, which is a major cause of inflammation as a result of the imbalance between ROS production and their removal by antioxidants. These findings imply that LCN2 may play a substantial role in iron-related oxidative stress damage and in inflammation.

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Conflict of Interest

The authors have no conflicts of interest regarding the publication of this article.

Adherence to Ethical Standards

This study has received ethical approval from the Medical Research Ethics Committee in the Iraqi Ministry of Health - Nineveh Health and the University of Mosul. The study approval number and date (2467 on 29 /3/2022).

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