EFFECT OF OMEGA-3 ON CARDIAC MUSCLE STRUCTURE IN A MODEL OF RHEUMATOID ARTHRITIS IN RAT. A LIGHT AND ELECTRON MICROSCOPIC STUDY

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ABSTRACT
Background: Rheumatoid arthritis (RA) is the most common form of chronic inflammatory arthritis. High percent of premature mortality in RA patients was largely attributable to cardiovascular diseases. Unfortunately, no curative treatment was found for this disease yet. Omega-3 polyunsaturated fatty acids (PUFAs) were largely studied for their anti-inflammatory effects. Aim of the work: to evaluate the effect of omega-3 PUFAs on the myocardial structure of left ventricle (LV) in a model of Adjuvant-induced RA in rats. Material and methods: Thirty rats were divided into three groups; control group, RA group and omega 3-treated group. RA was induced by single subcutaneous injection of complete Freund adjuvant in hind paw of rats. Omega-3 was orally administered after two weeks of RA induction, and continued daily for another two weeks. The ankle joint and the heart were dissected out after two and four weeks and processed for light microscopic examination. Specimens from the left ventricle were also processed for transmission electron microscopic examination. Histomorphometric studies and statistical analysis were also done. Results: RA was confirmed histologically in the ankle joint after two weeks. The LV also showed focal degeneration of the myocardium, and mononuclear cellular infiltration that was more evident after four weeks of untreated RA. Omega-3 treated group showed significant decrease of myocardial structure damage. Conclusion and recommendations: Omega-3 significantly ameliorated the structural damage of LV myocardium after RA induction. It is highly recommended to use omega 3 PUFAs regularly as early as possible in RA cases.

KEYWORDS: rheumatoid arthritis, cardiac muscle, omega 3, rats, complete Freund Adjuvant.

INTRODUCTION
Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease of unknown etiology primarily affecting the connective tissue. It mainly targets joints causing chronic, progressive, erosive and destructive polyarthritis.1 Systemic and extra-articular manifestations of RA were detected in high percentage of population having this pathology. Most importantly, the increased cardiovascular disease (CVD) in patients with RA is now a well-established problem[2] that was recently considered to be the leading cause of premature mortality in RA patients.3 The CVD risk seems to increase soon after RA diagnosis[4] and become evident within one year of its clinical onset.[5] Increasing risk of stable angina, myocardial infarction, heart failure and stroke were demonstrated in RA patients.[6] In addition, subclinical involvement was recently thought to be higher than anticipated.[5] Unfortunately, a permanent cure for RA disease is not yet available, therefore, its treatment remains problematic. It is currently treated mainly by disease-modifying antirheumatic drugs (DMARDs), such as methotrexate and sulfasalazine, and nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs must be also combined with steroid hormones like cortisone and prednisone.7 However, these regimens are not curative, they only transiently suppress inflammation and ameliorate symptoms.[8] Moreover, their other effects, as in the case of steroids, may increase heart disease risk.[9] Hence, other drugs with high efficacy and less toxicity are highly needed.

Omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids that possess the most potent immunomodulatory activities. Among the omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are more biologically potent than alpha linolemic acid (ALA). The anti-inflammatory properties of omega-3 PUFAs were well documented in many chronic inflammatory conditions as inflammatory bowel diseases,[10] kidney failure[11] and respiratory diseases.[12] Additionally, their anti-arrhythmic, anti-thrombotic, anti-atherosclerotic effects were demonstrated.[13]
Complete Freund’s Adjuvant (CFA), is the most widely used method for induction of autoimmune disease in rodents.\[^{14}\] It mimics the immune activation pathways elicited by infectious agents\[^{15}\] and exhibit many similarities to human RA.\[^{16}\] Thus, the present study aimed to evaluate the histological effects of adjuvant-induced rheumatoid arthritis on the myocardial structure of the LV. In addition, to histologically assess the therapeutic potential of omega-3 PUFAs.

**MATERIALS AND METHODS**

Thirty adult male albino Wistar rats weighing 180-200 gm were used in this study. Animals were purchased from and housed in the Medical Research Center, Faculty of Medicine, Ain Shams University, Cairo, Egypt. They were housed under standard conditions of housing and were put in clean wire mesh cages with free access to standard chow diet and tap water. Animals were carefully selected free of gait disturbance, swollen joints or limp. All the experimental procedures were carried out according to the recommendation and the guidelines of the institutional animal ethics committee at Faculty of Medicine, Ain Shams University. After seven days of acclimatization, animals were randomly divided into three groups.

**Group I: (Control group)**

This group consisted of fifteen rats, which were equally divided into two subgroups as follows:
- **Subgroup (IA):** rats (n=10) received a single subcutaneous injection of 0.2 ml normal physiological saline (0.9% NaCl) in the metatarsal footpad of right hind paw using a 1 ml syringe (Ameco, Egypt, Tenth of Ramadan city, Egypt). The animals were then divided equally to be sacrificed after two and four weeks respectively.
- **Subgroup (IB):** rats (n=5) received subcutaneous saline injection in the same site and dose as in subgroup IA. Then, after two weeks of induction, they received daily omega-3 in a dose of 300 mg/kg, orally by intragastric tube for two weeks, after which they were sacrificed (fourth week of the experiment).

**Group II: (RA group)**

Animals of this group (n=10) served as a rheumatoid arthritis (RA) model. Induction of RA was done by single subcutaneous injection of 0.1ml of complete Freund adjuvant (CFA) suspended in 0.2 ml normal saline in the metatarsal footpad of right hind limb using 1 ml syringe.\[^{17}\] The CFA was purchased as non-metabolizable oils (paraffin oil and mannide monooleate) containing one mg/ml heat-killed and dried Mycobacterium tuberculosis (Sigma- Aldrich, St. Louis, MO, USA). The animals were then equally divided into two subgroups:
- **Subgroup IIA (Early RA):** Rats were left untreated and sacrificed after two weeks to confirm the occurrence of RA.
- **Subgroup IIB (progressive RA):** rats were left untreated and were sacrificed after four weeks.

**Group III: (Omega-3-treated group)**

Rats of this group (n=5) were treated with oral omega-3 once daily by an intra-gastric tube, at a dose of 300 mg/kg (equivalent to 0.02 ml fish oil/rat).\[^{18}\] The treatment started after two weeks of RA induction as in group II and continued for another two weeks, after which the rats were sacrificed (four weeks from the beginning of the experiment). Omega-3 was used in the form of fish oil-containing gelatinous capsules, containing 300 mg omega-3 fatty acids; EPA+DHA, (Kirkland signature dietary supplements). Each capsule was carefully evacuated by a 1 ml syringe and given to rats as stated.

The day of injection was considered day zero for all groups. All injections were preceded by sterilization of skin of right hind paw with Betadine antiseptic solution. The rats were anaesthetised by ether inhalation to be sacrificed at the appropriate timing. The right ankle joint and the heart were dissected out in each time point.

**Ankle joint processing**

The right ankle joints were fixed and decalcified in the chelating agent ethylene-diamine-tetra-acetic acid (EDTA) in the form of its disodium salt (5.5 g EDTA in 90 ml distilled water and 10 ml formaldehyde, 37.40%). This solution was renewed every other day for one month, after which the specimens were completely soft and decalcified. The decalcified joints were cleaved longitudinally in a sagittal plane along the central line and were then processed to obtain paraffin blocks, cut into serial five micrometer-thick sections and stained by H&E.

**Heart tissue processing**

The heart was longitudinally cut and part of the left ventricle was fixed directly in 10% neutral buffered formalin, to be further processed into paraffin blocks and subsequently cut into five micrometer-thick sections. These sections were stained by H&E and Masson’s trichrome stain.\[^{19}\] Small one mm² pieces of the LV wall were also cut, fixed in 2.5% glutaraldehyde to be processed for transmission electron microscopic examination.\[^{19}\]

**Morphometric study and statistical analysis**

The area percentage of collagen fibers was measured in Masson’s trichrome-stained heart sections. This was done in five non-overlapping stained sections (five high-power fields, 40x / section) from all the rats in each group. Quantitative measurements were performed using the image analyzer Leica Q win V.3 program installed on a computer connected to Leica DM2500 microscope (Wetzlar, Germany) in the Histology Department, Faculty of Medicine, Ain Shams University. Mean±SD were calculated. The SSPS program version 21 (IBM Inc, Chicago, Illinois, USA) was used to analyze the
differences among all groups in all the data parameters by one-way analysis of variance and a post-hoc test. The measurements were considered significant if P value was ≤ 0.05.

RESULTS

A- Ankle joint

The H&E-stained sections of the control animals in both subgroups IA and IB showed the tibial end and talus bone of the ankle joint covered by articular hyaline cartilage with regular smooth surface, lacking the covering perichondrium. The superficial tangential zone of the articular cartilage comprised flat chondrocytes parallel to the surface. The middle transitional zone contained larger and more rounded scattered chondrocytes. The radial zone consisted of short rows of rounded chondrocytes perpendicular to the surface. The chondrocytes were located individually inside their lacunae, appeared with pale basophilic cytoplasm and central rounded vesicular nuclei and were rarely seen forming cell nests. A zone of calcified cartilage containing scattered chondrocytes with pyknotic nuclei and located inside their lacunae, was seen separating the radial zone from the underlying subchondral bone (Fig.1a). The synovial membrane consisted of one or two layers of intimal cells with an underneath subintimal layer and a connective tissue stroma. Two types of synovial intimal cells were detected; macrophage-like cells (type A synoviocytes), with irregularly rounded or oval nuclei and basophilic cytoplasm and fibroblast-like synovial cells (type B synoviocytes) with flattened nuclei and basophilic cytoplasm. The synovial subintimal layer and stroma consisted of fibroadipose connective tissue with predominate fat cells and some regularly arranged collagen fibers (Fig.1b).

In subgroup IIA (after two weeks of RA induction), the H&E-stained sections confirmed the occurrence of RA by showing surface irregularities and focally eroded areas in articular cartilage of talus bone. These erosions reached the calcified cartilage layer and were covered by part of periosteum containing mononuclear inflammatory cells, probably migrating from the periphery of the joint. Degenerated chondrocytes with pyknotic nuclei were seen underneath and around the eroded areas (Fig.1c). Clusters of mononuclear cells including Antischkow cells were also seen in the stroma of the synovial membrane. Antischkow cells had basophilic cytoplasm and were seen either longitudinally cut exhibiting single central caterpillar-like longitudinal nuclei, or transversely cut with single central rounded nuclei resembling Owl’s eye. These aggregations were often seen in the vicinity of dilated congested blood vessels with focally disrupted endothelium. Hyaline acidophilic material was observed in the dilated blood vessels (Fig. 1d).

In subgroup IIB (progressive RA, four weeks after RA induction), massive articular cartilage erosions exposing the subchondral bone of talus was evident, leaving small areas of covering cartilage at the periphery. These erosions were also seen in margins of tibia, however, they were superficial reaching only the transitional zone (Fig.1e). Apparently increased number of macrophage-like type (A) synoviocytes was seen in focal areas of synovial membranes’ intima. Mononuclear cellular aggregations including Antischkow cells were also seen in the stroma of the synovial membrane closely related to congested synovial blood vessels (Fig.1f).

Omega-3 administration in group III showed improvement of the ankle joint structure. Smooth surface and regular structure of ankle joint articular cartilage was seen. Moreover, the chondrocytes’ structure and organization appeared comparable to that of the control group (Fig. 1g), as well was the structure of the synovial membrane. However, small aggregations of mononuclear inflammatory cells were seen in its stroma (Fig. 1h).

![Figure 1: Ankle joint structure. Group I: control group (a &b). (a): Normal organization and regular surface of articular cartilage of talus bone. Chondrocytes in tangential (†), transitional (▲), and radial (△) zones can be seen. Notice the calcified cartilage zone (C), and the subchondral bone (B). (b): macrophage-like cells (▲) and fibroblast-](image-url)
like cells (†) of synovial membrane intima. A blood vessel (V) can be seen in the synovial membrane stroma. **Subgroup IIA; early RA (c & d). (c):** Focal area of articular cartilage erosion reaching the calcified cartilage zone (Δ) covered by part of periosteum containing inflammatory cells (▲). Notice the degenerated chondrocytes (†). (d): Clusters of macrophages are seen in the synovial membrane (†). Notice dilated congested blood vessel (V), with focal disruption in its endothelium (Δ). Inset shows transversely (†) or longitudinally cut (▲) Anitschkow cells. **Subgroup IIB; progressive RA (e & f). (e):** Massive erosion of articular cartilage of talus (Δ), and partial erosion in peripheral part of tibial end (†). Part of articular cartilage is seen covering peripheral part of talus (▲). (f): Apparently increased number of macrophage-like synoviocytes type A (Δ), and mononuclear cellular aggregations (▲) in stroma of synovial membrane, in relation to congested vessels (V). Inset shows Owl’s eye appearance of Anitschkow cells (†). **Group III; Omega-3 treated group (g & h). (g):** Smooth surface (▲) and regular structure of articular cartilage covering the talus. Chondrocytes are seen with vesicular nuclei inside their lacunae (†). (h): Small aggregations of mononuclear inflammatory cells in synovial membrane (†). 

**H&E; all x 400, except (e) x 100, insets x 1000**

**B- Left ventricle myocardium**

All subgroups of the control group showed the same histological picture.

**I- H&E results**

The H&E-stained sections of the LV myocardium showed branching and anastomosing cardiac muscle fibers running in different directions (Fig. 2a). Cytoplasmic striations were seen in the cardiomyocytes, and their nuclei appeared central, oval, and vesicular. Fibroblasts with their elongated nuclei were seen in-between the myocardial fibers (Fig. 2b).

The H&E-stained sections of LV of myocardium subgroup IIA (Early RA) showed cardiomyocyte degenerative changes in the early stages of the disease. Focal degeneration and fragmentation of some myocardial fibers was seen. Patchy areas were seen with pale stained myocardial fibers devoid of nuclei. Few fibers appeared with deeply acidophilic cytoplasm, small deep densely stained pyknotic nuclei and absent striations (Figs. 2c, 2d). No obvious regular striations were seen in most of the cardiomyocytes, however, regular striations were retained in others. Apparently wide edematous interstitium was noticed in-between the cardiac muscle fibers which appeared separated in multiple areas (Fig. 2d). Some mononuclear cells were observed in-between the myocardial fibers (Fig. 2e), together with congested dilated blood vessels (Figs. 2c, 2d). Acidophilic homogenous hyaline exudate was seen together with extravasated red blood cells (RBCs) in-between the fragmented cardiomyocytes (Fig. 2c). Disrupted endothelial lining of coronary vessels could be observed, together with few mononuclear cells in their walls (Fig. 2e).

In subgroup IIB (progressive RA), the H&E-stained sections of the left ventricle showed progression of the RA-induced myocardial damage. Focal cardiomyocytes degeneration, with diffuse infiltration by mononuclear cells were evident in many sites in the left ventricle (Figs. 2f, 2g). Most of the cardiomyocytes were seen with pale cytoplasm and devoid of nuclei, and appeared thinner as compared to those of the control group. Fragmented cardiomyocytes were also noticed (Fig. 2f). The mononuclear cellular infiltrate was seen mainly formed of abundant lymphocytes with their rounded darkly stained nuclei surrounded by scant basophilic cytoplasm. Groups of multiple large oval or rounded Anitschkow cells were clearly identified (Figs. 2f, 2g). These cells were often seen either in small groups in-between the cardiac muscle fibers (Fig. 2f), or forming multiple variable-sized nodular-like aggregations often disrupting the cardiomyocytes’ organization (Fig. 2g), displacing and sometimes replacing, the cardiomyocytes at focal points. Moreover, these mononuclear cells were commonly found in the vicinity of dilated congested blood vessels of synovial stroma (Figs. 2f, 2g).

Omega-3 administration in group III resulted in notable improvement of the myocardial structure. Most of the cardiomyocytes appeared comparable to the control group. However, few cardiomyocytes appeared with deeply acidophilic cytoplasm. Small congested blood vessels were noticed in-between the cardiomyocytes in focal areas, together with few mononuclear inflammatory cells (Fig. 2h).
Figure 2: Cardiac muscle structure. Group I; control group (a & b). (a): branching and anastomosing cardiac muscle fibers of LV myocardium running in different directions (↑). (b): Transverse striations can be seen in the cardiomyocytes (△). Notice the oval vesicular nuclei of cardiomyocytes (↑), and the elongated nuclei of fibroblasts (▲). Subgroup IIA; early RA (c, d, e). (c): Fragmented cardiomyocytes can be seen (F). Notice cardiomyocytes devoid of nuclei (↑↑), or with pyknotic nuclei (↑). A cardiomyocyte with deeply acidophilic cytoplasm can be seen (▲). Mononuclear cells (△), and dilated congested blood vessel can be noticed (*). (d): Few fibers exhibiting regular cytoplasmic striations (↑↑). Eosinophilic exudate can be seen (▲), with extravasated RBCs (△). Fragmented cardiomyocytes are observed (F). Notice pyknotic nucleus (↑), and dilated congested blood vessel (*). (e): Coronary vessels with disrupted endothelial lining and few mononuclear cells. Subgroup IIB; progressive RA (f & g). (f): Focal area of degenerated cardiomyocytes (D), and mononuclear cellular infiltration (△). Notice fragmented (↑) or thinned out cardiomyocytes (▲). Inset shows high magnification of caterpillar-like Anitschkow cells (↑↑). (g): Thinned out cardiomyocytes can be seen (↑↑). Groups of mononuclear cells (I) are seen replacing or displacing the cardiomyocytes. Notice caterpillar-like Anitschkow cells (△). Inset shows high magnification of Anitschkow cells either transversely cut (↑), or as longitudinal caterpillar-like cells (△). The presence of lymphocytes (▲). Group III; omega-3 treated group (h): Most cardiomyocytes are seen with vesicular nuclei (↑) and few with deeply acidophilic cytoplasm (*). Small congested blood vessel (△) and few mononuclear cells can be noticed (▲). H&E, 2a x100, 2b-2h x 400, insets x1000
**II- Masson’s trichrome stained section**

Minimal collagen fibers were observed between the cardiac muscle fibers of the left ventricle of the control group (Fig. 3a). The collagen area percentage was measured as (1.50±0.54, mean ±SD, Histogram 1).

In subgroup IIA (early RA group), some collagen fibers were seen in-between the cardiomyocytes (Fig. 3b). Significantly increased collagen area percentage was statistically detected as compared to the control group, measuring (21.49±0.56, P<0.05, Histogram 1).

The Masson’s trichrome-stained sections of subgroup IIB (progressive RA group), showed abundant collagen fibers (Fig. 3c). The collagen area percentage measured (56.79±0.97), which was significantly increased (P<0.05), as compared to group I and subgroup IIA (table & histogram 1).

Omega-3 administration in group III, showed few collagen fibers in-between the cardiomyocytes (Fig. 3d). The collagen area percentage measured (2.53±0.33), which was significantly decreased (P<0.05) as compared to subgroups of group II; IIA and IIB. However, this value was significantly increased (P<0.05) as compared to the control group (Histogram 1).

**Figure 3: Collagen fibers content in cardiac muscle. Group I; control group (a): Minimal content of collagen fibers can be seen in-between the cardiomyocytes (↑). Subgroup II; early RA (b): Some collagen fibers are seen in-between the cardiomyocytes (↑). Subgroup IIB; progressive RA (c): Abundant collagen fibers are seen in-between the cardiomyocytes (↑). Group III; omega-3 treated group (d): Few collagen fibers are seen in-between the cardiomyocytes (↑). Masson’s trichrome x 400**

**Histogram (1): Showing the area percentage of collagen fibers in different groups:**

- Values are expressed as mean±SD
- #: Significance calculated by least significant difference (LSD) at P<0.05 from the control group (I).
- $: $ Significance calculated by LSD at P<0.05 from subgroup (IIA).
- **#:** Significance calculated by LSD at P<0.05 from subgroup (IIB).

**III- Transmission Electron microscopic results**

Group I (the control group) showed regularly arranged myofilaments between successive Z lines in the sarcomeres. The H zone with central M line were also...
clearly identified in the middle of each sarcomere. The cardiomyocytes’ nuclei appeared regular with dispersed euchromatin. Abundant mitochondria, with apparent cristae, were seen regularly arranged in-between the myofibrils. Step-like intercalated discs appeared in-between the adjacent cardiac muscle fibers (Fig. 4a).

Subgroup IIA (early RA) showed disrupted myofilaments’ organization, appearing separated and fragmented. Most of the mitochondria appeared variable in size, distorted with electron dense matrix and unapparent cristae. Widening of the intercalated discs was noticed (Fig. 4b).

Subgroup IIB (progressive RA) showed widely-spaced myofibrils that appeared fragmented inside the cardiomyocytes. Disorganized degenerated mitochondria, mostly with unapparent cristae were seen in-between the fragmented myofibrils (Figs. 4c, 4d). The nuclei were seen either euchromatic with irregular nuclear envelope (Fig. 4c), or apoptotic appearing small irregular and heterochromatic. Incomplete, disrupted intercalated discs were observed between cardiac muscle fibers (Fig. 4d).

Omega-3 administration in group III showed almost regular arrangement of most of myofibrils. Few disrupted myofilaments were observed. The mitochondria appeared regularly arranged between the myofibrils, however, with they appeared with electron dense matrix and unapparent cristae. The intercalated discs retained their step-like appearance between cardiomyocytes (Fig. 4e).

Figure 4: Cardiac muscle ultrastructure. Group I; control group (a): A cardiomyocyte with regularly-spaced myofibrils. Notice the (Z) lines (Δ) and (M) lines in the center of (H) zones (†) and mitochondria (M). The inset shows step-like intercalated disc (yellow Δ). Subgroup IIA; early RA (b): Disrupted (Δ), and separated (S) myofilaments can be seen. Notice the distorted mitochondria (†), and the widened intercalated disc (yellow Δ). Subgroup IIB; progressive RA (c & d). (c): A cardiomyocyte showing disrupted organization of myofibrils. Fragmented myofibrils can be seen (Δ). Notice the irregular nuclear envelope (N), and the degenerated disorganized mitochondria (†). (d): Apoptotic nucleus (N) can be seen among fragmented myofibrils (*). Notice degenerated mitochondria (†), and disrupted intercalated disc (yellow Δ). Group III; omega-3 treated group (e): Regularly arranged myofibrils with intact organized myofilaments (†). Notice the regularly arranged mitochondria with unapparent cristae (*), and the step like intercalated disc (yellow Δ).

TEM, (a) x 20000, Inset x 20000 - (b & inset) x 25000- (c) x 10000 - (d) x 20000, inset x 25000 – (e & inset) x 25000).
DISCUSSION
Complete Freund’s Adjuvant (CFA) used in the present study is considered to be the most effective adjuvant available. It is the most widely used method to induce an autoimmune disease in rodents, mirroring much of the pathology of RA.[14]

In the present study, RA was confirmed histologically in H&E-stained sections of group (IIB) and progressed after four weeks of RA induction in group (IIB). Partial resorption of articular cartilage of the ankle joint was detected after two weeks of RA induction. It was more evident after four weeks, with hyperplasia of the macrophage-like cells type (A) of synovial membrane. Mononuclear cellular infiltration was seen aggregated in the stroma of synovial membrane. These findings were aggravated after four weeks of untreated RA. This agreed with previous literatures which confirmed that RA development after two weeks[20] and progressed after four weeks of RA induction.[21]

Results of H&E staining and of TEM examination of the left ventricle myocardium in the present study correlated with the findings of ankle joint and confirmed the presence of LV focal structural damage after RA induction. Degeneration and necrosis of cardiomyocytes in subgroup IIA (early RA) was detected and was more evidently seen in subgroup IIB (progressive RA). These findings were in agreement with several investigations linking RA to cardiovascular diseases in general[22, 23] and to the left ventricle myocardium affection in particular.[24] Wide interstitium and separation of the cardiomyocytes were seen in the present study. This can be attributed to disrupted intercalated discs that was noted in both subgroups IIA and IIB of the present study by TEM.

Aggregations of Anitschkow cells were seen in the present study in synovial membrane in subgroups IIA and IIB and in-between the cardiomyocytes after RA progression in subgroup IIB. These granulomatous aggregations were considered specific for RA, sharing the same morphological features with subcutaneous RA nodules with predilection for the myocardium of the left ventricle.[25] These cells were stated to be enlarged histiocytes that developed prominent elongated nuclei and nucleoli resembling caterpillar body, hence their other name ‘caterpillar cells’. They usually infiltrate tissues in groups called ‘Aschoff bodies’ seen as nodules in close association with small blood vessels and their presence was considered pathognomonic to rheumatic diseases.[26] These cells were also found separately among other mononuclear cells in the present study. These scattered cells were reported to be a non-specific form of RA morphologically characterized by interstitial infiltration with lymphocytes, plasma cells and histiocytes.[25]

In addition, lymphocytes were also numerous seen in left ventricle myocardium in the present study, especially in the progressive RA subgroup IIB. In this regard, activated T-lymphocytes were demonstrated as the most important contributing factors in the pathogenesis of RA.[27] Emerging data confirmed that T lymphocytes play a crucial pathogenic role in both RA[26] and heart disease.[29] Experimental autoimmune myocarditis was reported to be driven mainly by T lymphocytes[30], which were reported to be abundantly present in peripheral blood of Patients having RA.[31]

Previous researches reported that the same pathogenic mechanisms of joint inflammation contributed to increased cardiovascular diseases in RA patients.[32] Several mechanisms were implicated in the pathology of RA, that might simultaneously lead to myocardial structural changes. Initially, immune complex formation appeared to be an important step allowing pathogenic autoantibodies access to the synovium.[33] These blood-circulating immune complexes might lead to increased vasopermeability especially in the distal extremities, allowing influx of inflammatory cells into the joint tissue.[34] This might explain the presence of inflammatory exudate and congested dilated blood vessels in the synovial membrane after two weeks of RA induction in the present study. Moreover, this immune complex deposition co-localizes with complement component eventually resulting in ongoing synovitis.[35] Synoviocytes were implicated as a key factor in the pathogenesis of this destructive autoimmune chronic inflammatory disease, and might be also a primary target for the initial immune process in RA.[36] This correlates with the results of the present study that showed synovial cells hyperplasia, together with synovitis and myocarditis. It was demonstrated that in RA, macrophages, fibroblast-like synoviocytes and activated T cells produce pro-inflammatory mediators, such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), which play key roles in the pathogenesis of RA.[36] These cytokines might be involved in promoting inflammation, synovial tissue hyperplasia, osteoclast differentiation and invasion of the periosteal surface adjacent to articular cartilage.[37] In this context, IL-1β and TNF-α might stimulate osteoclasts and chondrocytes to release the cartilage and bone-destroying metalloproteinases.[37] In addition, interleukin-6 was reported to promote local leukocyte activation and autoantibody production. It was considered as an important mediator of the systemic effects of RA patients.[38] This might explain erosion of the articular cartilage detected in the present study in both subgroups IIA and IIB.

Furthermore, TNF-α was reported to contribute to the endothelial dysfunction with increased expression of adhesion molecules. Endothelium was also implicated in the expression of major histocompatibility complex (MHC) class II, that was reported to one of the causes of endothelial dysfunction. Moreover, the endothelial expression of this MHC class II might attribute to the activation and migration of T- cells and monocytes into
the vascular wall. This is going with the presence of extravasated RBCs found in both subgroups of RA in the present study. This might also contribute to the partial loss of endothelium of coronary vessels and presence of inflammatory cells in their wall seen in early RA subgroup IIA of the current study.

The results of the current work showed mitochondrial damage in early and late RA in subgroups IIA and IIB respectively. This might be explained by accumulation of reactive oxygen species (ROS), as oxidative stress was also reported to play a crucial role in the pathogenesis of RA and its associated myocardial structural changes. Free radicals and ROS along with oxidants produced by macrophages and neutrophils, were accused for chronic oxidative stress in the RA synovial microenvironment and the other tissues. In addition, via production of 4-hydroxynonenal (HNE), ROS might damage cellular components by direct oxidation, damaging the mitochondrial energy metabolism. Compromising mitochondrial energy metabolism might easily contribute to the development of cardiac hypertrophy and its progression to heart failure. Thus, HNE was used as a biomarker of oxidative stress-induced lipid peroxidation, however, it was also involved in cell proliferation, apoptosis, and inflammation, which are hallmarks of cardiovascular diseases.

Significant fibrosis of the myocardium was detected by Masson’s trichrome stain in the present study in subgroups IIA and IIB. This agreed with the results of previous research which demonstrated increase cardiac fibrosis in LV tissues of adjuvant induced RA in rats. These researchers proposed that increased HNE levels in the heart of RA rats would play a key role in the pathogenesis of fibrosis. Pro-inflammatory cytokines, secreted from macrophages, such as TNF-α and IL-1β, were also suggested to cause cardiac remodeling and myocardial fibrosis. Moreover, transforming growth factor-beta (TGF-β) was strongly suggested to as profibrotic agent by induction of the pro-inflammatory cytokines matrix metalloproteinases (MMPs) degradation or remodeling processes in RA.

Administration of omega-3 in the present study had a significant therapeutic effect on the both; the ankle joint and left ventricle myocardial structure. Animal experiments also demonstrated benefit from omega-3 fatty acids in models of RA, as they delayed the onset of arthritis, reduced its severity and improved joint pathology. These results were also in agreement with previous researches that established the cardiovascular benefits of omega-3 supplementation. Other study showed the improvement of the mechanical function of the heart using omega-3 fatty acid supplements, in addition to its association with lower incidence of heart failure.

It was reported that due to its high concentration of eicosapentaenoic and docosahexaenoic acids, omega-3 fish oil has been known by its anti-inflammatory potential. These fatty acids were able to partly inhibit a number of aspects of inflammation. Strong anti-inflammatory effect of Omega-3 parenteral nutrition was detected in liver disease. The authors documented that omega-3 decreased proinflammatory cytokines including IL-1β, IL-6 and TNF-α. In addition, omega-3 PUFAs were found to produce immunomodulatory eicosanoids such as prostaglandins, thromboxanes and leukotrienes through direct effects on their transcription regulation. Investigators also attributed the anti-inflammatory action of omega-3 PUFAs to their production of the lipid mediators resolvins and protectins. Resolvin was reported to inhibit IL-1β production, likewise, protectin inhibited tumor necrosis factor IL-1β, and TNF-α production. In this context, resolvins were reported to reduce inflammation and protect experimental animals in models of inflammatory disease including arthritis. Moreover, researchers have also reported that omega-3 PUFAs reduced T cell proliferation. Additionally, they disrupt lipid raft chemical composition of T-lymphocyte, which are intimately involved in these cells’ response to activation, thus altering their functioning.

The anti-fibrotic of omega-3 PUFAs were also reported. Specifically, its docosahexaenoic acids fully inhibited TGF-β-induced epithelial mesenchymal transition process in liver and prostate. Moreover, it also decreased matrix metalloproteinase-9 (MMP-9) in serum and in stimulated monocytes thus reduced monocyte liver infiltration and development of liver fibrosis. Thus, it could also be suggested that through its inhibitory action on TGF-β, omega-3 PUFAs were able to significantly decrease fibrosis in group III of the present study.

Taken together, it can be concluded that RA induction in the present study, not only affected the ankle joint, but also led to an extra-articular manifestation in the form of structural damage in the left ventricular myocardium. On the other hand, early omega-3 administration, two weeks after RA induction, has evidently managed to reverse the myocardial structural changes by exerting anti-inflammatory and anti-fibrotic effects. It is highly recommended to use omega 3 PUFAs regularly as early as possible in RA cases in order to protect against this disease’s deleterious myocardial structural changes. Further studies on the effect of omega-3 on other organs are recommended to fully elucidate its potential therapeutic role in autoimmune diseases.

Conflict of interest
No conflict of interest to declare.

REFERENCES


