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THESIS

THE EFFECT OF ANTI-OXIDANT (VITAMIN E AND C) ACTING AS ANTI-INFLAMMATORY IN OSTEOARTHRITIS PATIENT TREATED WITH DICLOFENAC SODIUM

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 14.04.2015 and numbered 2015/10-1

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

To my country and my family ...

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List of Abbreviations

OA……Osteoarthritis

RA…….Rheumatoid Arthritis

IL……..Interleukins

MDA……Malondialdehyde

GSH……..Glutathione

SOD……..Superoxide dismutase

CP…….Ceruloplasmin

TF……Transferring

ESR……Erythrocyte sedimentation rate

CRP……C-reactive protein

RF……..Rheumatoid factor

Hgb……..Hemoglobin

WBCs……..White blood cells

NSAIDs……...Nonsteroidal anti-inflammatory drugs

GIT…….Gastrointestinal tract

COX……..Cyclooxygenase

GS…….Glucosamine sulphate

CS……...Chondrpitin sulphate

GAG……Glycosaminoglycan

MSM…… Methylsulphonyl methane

DLPA……D-L-phenylalanine

SAMe…….S-adenosyl methione

ROS……..Reactive oxygen species

H2O2……Hydrogen peroxide

HOCl…….Hypochlorous acid

O2-…….Superoxide radical

.OH……...Hydroxyl radical

ROO……...Aproxyl radical

1O2……..Singlet oxygen

NO……Nitric oxide

ONOO-……..APeroxyl nitrite

FRs……..Free radicals

ESR…….Electron spin resonance spectroscopy

EPR……Electron paramagnetic resonance

XPS……X-ray photoelectron spectroscopy

GSSG……..Oxidized glutathione

IU……..International unit

ATP……Adenosine triphosphate

DHA……Dihydroxy ascorbic acid

SLE……...Systemic lupus erythmatosus

IgG, M, A……Immunoglobulins, G, M, A

RBCs……...Red blood cells

PCV……..Packed cell volume

KH2PO4…….Potassium dihydrogen phosphate

Na2HPO4……...Disodium hydrogen phosphate

DTNB……Dithionitrosobenzoic acid

SSA……..Sulphasalicylic acid

TCA……Trichloroacetic acid

TBA……..Thiobarbituric acid

DMARDs……...Disease modifying antirheumatoid drugs

PMHx……..Past medical history

FHx……...Family history

BMI……..Body mass index

KOOS……..Knee injury and osteoarthritis outcome score

Abstract

Background

OA it's a common disease that occur in elderly people and it its most come in the female under age 49 than a male above 50 because of the hormonal changes and for the men for aging and other chronic disease.

Objective

Our objective was directed to determine the beneficial effect of the antioxidants (vitamin E and C) as anti-inflammatory in OA patients treated with diclofenac sodium.

There is a reality that oxidative stress give a good progression and incidence of osteoarthritis (OA), increased production of reactive oxygen species (ROS) and decreased bioavailability of antioxidants have been checked by experimental and human OA, where increased ROS production may contribute to the elevation in OA incidence. and we use in this study different ages with OA patient to can determind the best reslu and effect we can get from this study.

Patients and methods

The ESR, Hgb, and serum (CRP, RF, MDA, and GSH) level were measured for each patient before treatment (at the baseline) and after 3, 5, 7, and 8 weeks of the treatment with documentation of symptoms and pain score before and after treatment (the treatment were continue for 2 months).

Results

There was significant ($p < 0.05$) elevation in serum MDA level and significant reduction in serum GSH level in OA patients as compared to the healthy control subjects. The addition of vitamin E to diclofenac sodium (group B) had resulted in a significant lowering in serum MDA level with significant elevation in serum GSH level and OA outcome score (symptoms and signs score) after 3, 5, 7, and 8 weeks of treatment, likewise the addition of vitamin C to diclofenac sodium (group C) with less positive effect than vitamin E, while the use of combined antioxidants vitamin E and C (group D) resulted in significant lowering in serum MDA level with significant elevation in serum GSH level and OA outcome score (symptoms and pain score) after 3, 5, 7, and 8 weeks of the treatment with great positive effect than the use of single antioxidant (vitamin E or C) due to synergistic effect.

Conclusion

The oxidative stress give a progression of OA and the addition of antioxidants (vitamin E or C) to diclofenac sodium treatment give a good lowering in MDA level, and elevation in GSH level and OA outcome score (symptoms and pain score) with improving the patients compliance but all the result that I got it gave a negative result with African races .

Note. that test and that laboratory data I got it from the patient directly or from their physician after I get from them official agreement that was a proved by their physician so I got a full agreement to get a full excess for their laboratory data.

Osteoarthritis (OA)

1.1 Introduction

Osteoarthritis (OA) also known as degenerative arthritis, degenerative joint disease, wear and tear arthritis [1,2]. Is the most common form of arthritis that usually comes with age and most often affected the fingers, knees, and hips. Some times OA follow an injury to a joint [3,4]. OA is caused by wear and tear over time or by injury to the joint, which resulting in the degeneration of the hard and smooth layer of the cartilage that normally covers and protect the ends of the bones [5,6]. Unlike rheumatoid arthritis (RA), OA is not usually associated with activation of the immune system, so people with OA do not have the systemic symptoms of RA such as fever and fatigue that are caused by the release of the chemical messengers from the immune cells [5].

The usual symptoms of (OA) are deep aching pain which localized in the involved joint, stiffness after rest, joint swelling and tenderness, a grating sound when the joint is moved, and in the later stages bone deformities. The pain is usually present with movement of the joint and relieved by rest, it is rises in the joint capsule, ligaments, tendons, muscles, and bone surrounding the damaged cartilage, the affected joints may make a crunching noise as they move, this sound called (crepitus) result as the roughened articular surfaces rub together [1].

1.2 Prevalence and incidence

OA is responsible for more than 7milion physicians visit

per day, it is (8) times more common than RA, affect 20.7 millions of Americans, it almost affect every one over the age of 75 years at least one joint and common in women over age of 45 year and men below age of 45 year [7,8,9].

1.3 Joints often affected by OA

Joints often affected by OA are shown in figure (1.1).

A. Hands

The most commonly affected joints in the hands are the distal and proximal enter phalangeal joints, in which bony enlargement occurs which progresses slowly over many years and most often occurs in middle aged or elderly women, small gelatinous cysts may develop over the dorsal aspect of the distal enter phalangeal joints which either persist or resolve, many patients and Bouchard nodes have very little pain most of the time and therefore may not seek medical attention. While the carpometacarpal joint of the thumb is another frequently involved joint, either by itself or along with the distal joints; in such cases patients experience pain, bony enlargement, and limited motion of the thumb .

B. Knees

OA frequently affects the knees and may be a cause of significant disability (most patients with pain that worsens with activity and improves with the rest) patients with osteoarthritic knees always have crepitus, limited motion and pain while effusion may or may not be present, in more advanced disease bony enlargement, instability, and various angulation may be present.

C. Hips

Most patients experience a progressive disabling pain, usually in the upper thigh or inguinal region radiating to the knee, pain is worse with ambulation and might cause the patient to limp, patient may also complain of difficulty with activity such as tying shoes, and limited hip motion .

D. Spine

OA of the cervical and lumbar spine is referred to as spondylosis, involvement of intervertebral disk spaces may cause chronic back or neck pain that worsens with activity and improves with rest. In patients with extensive degenerative changes (fibrosis and osteophytes) stenosis of the spinal canal can occurs resulting in chronic cord compression in the cervical spine or compression of the cauda equine in the lumbar region, a variant of spinal OA occurring in the thoracic bridging and may cause loss of motion .

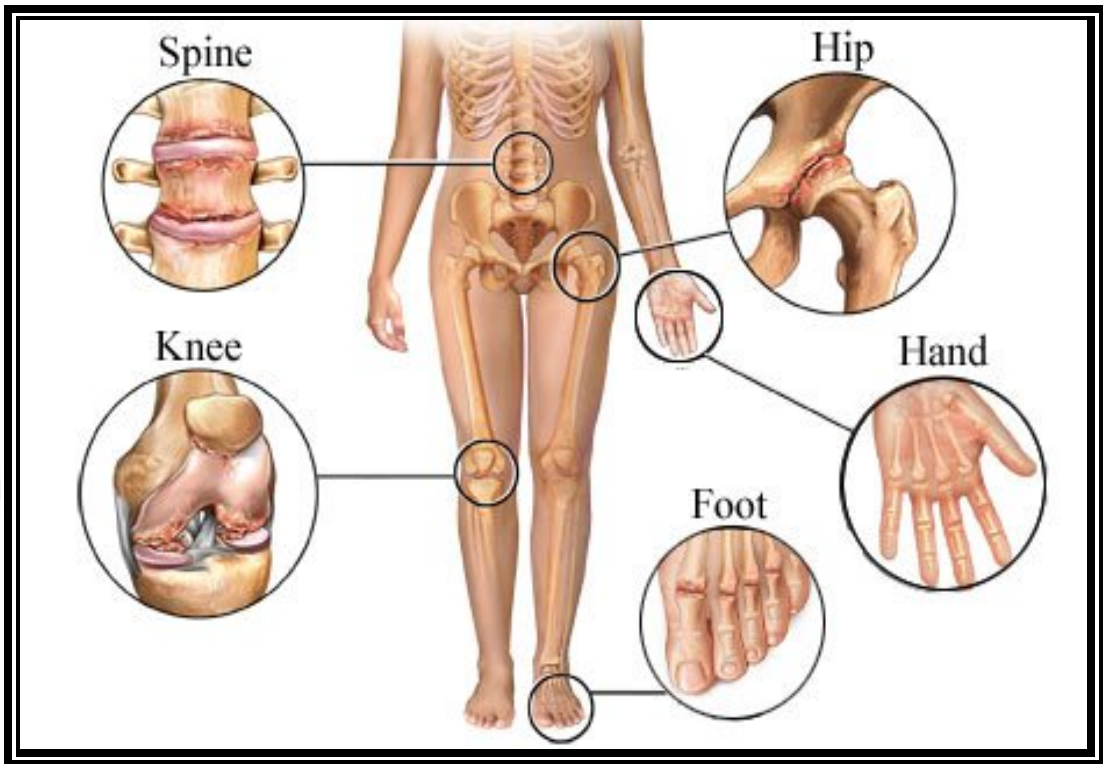


Figure (1.1): Joints often affected by osteoarthritis

1.2 Definition

OA is the most common form of arthritis (accounts for half of all cases) the word is derived from the Greek word "osteo" meaning " the bone", "arthro" meaning joint and "itis" meaning inflammation, although many suffers have little or no inflammation [(2,12)]. Is a condition in which the cartilage that protect and cushions the joint will breaks down over time and formation of new bone (osteophyte at the joint surface) [(4,13)]. Generally considered a non-inflammatory type of arthritis which referred to as degenerative joint disease or "wear and tear

arthritis" [1].

1.3 Classification

There are two categories of OA [7].

A. Primary OA

Primary OA appears to be associated with arthritis in patients without specific inflammatory or metabolic conditions, and patients who do not have a history of specific injury or trauma, the involvement is limited to a small number of joints, however in some patients multiple joints areas are involved [2,10].

B. Secondary OA

Secondary OA appear to be associated with several conditions that cause damage to articular cartilage through a variety of mechanisms including: mechanical, inflammatory, and metabolic process [2,10].

1.4 Epidemiology

OA is the most common type of arthritis and most common cause of disability [14,15], caused by wear and tear of the joint due to the natural result of aging [16]. The probability of OA increases with age including 80% of population over 50 years old, due to decrease ability of collagen matrix of the cartilage to repair itself [17].

1.5 Etiology

A number of factors are contributed to the development of OA.

A. Age

It is the factor that most strongly associated with radiographic and clinically significant OA, the cellular or

biomechanical changes in articular cartilage that occurs with aging may facilitate the development of the disease [18].

B. Obesity

There is positive association between OA of the knee and increased load carried by a person, this come from the alteration in gait and posture that redistribute this load, in addition to the deleterious effect of leptin on articular cartilage leading to structural damage [19,20]. A study in young men suggested that each increase in weight by 8 Kg result in a 70% increase in the risk of symptomatic arthritis of the knee in later years, also obese patients with joint misalignments are at a risk for more rapid progressive OA [21,22].

C. Bone density

Women with osteoporosis and hips fractures have decrease risk of OA and those affected by OA have significant increase in bone density, this negative association suggests that the soft subchonderal bone absorbs impact and protects articular cartilage better than dense bone [23,24].

D. Occupation

Increase occupational activities that require frequent knee bending, increase the risk of knee involvement and frequent lifting appears to be a risk factor for hip involvement [25,26].

E. Sports

Long term weight bearing sport activities are associated with an increased risk of developing radiographic evidence of OA [27,28].

F. Injury

Specific joint injury is an important risk factor for knee and hip OA [29].

G. Genetic factor

Hereditary factors are also important in OA of the hip joint as well as finger joints [30]

H. Metabolic abnormalities:

Metabolic abnormalities include genetic linkage to collagen genes, Oestrogen receptors, vitamin D receptors, and iron metabolism [31].

I. Hormonal imbalance:

OA increase after menopause due to Oestrogen deficiency [17].

J. Nutritional imbalance:

Research has shown that a diet rich in these antioxidants (vitamin E and vitamin C) can reduce inflammation of OA and slow the progression of the disease [32].

1.6 Pathophysiology

OA is a disease that affects all structures within the joint including articular cartilage, subchondral bone, ligaments, periarticulare muscles, and nerves. The normal articular cartilage is composed of sparsely scattered cells (chondrocytes) within an extra- cellular matrix composed of collagen, proteoglycans, and water with a very small component of calcium salts, collagen is arranged in thick bundles parallel to the surface of the cartilage, proteoglycan is composed predominantly of a large molecule called (aggrecan) which consist of large core protein with covalently attached side chain

of glycosaminoglycan's most of which are chondroitin sulfate and keratin sulfate [10].

In normal cartilage the turnover rate of collagen is relatively slow whereas proteoglycan turnover is rapid, the normal turnover of these matrix components is mediated by the chondrocytes which synthesize the components, and the proteolytic enzyme responsible for their breakdown, in turn chondrocytes influenced by a number of factors including cytokines, structural and physical stimuli, and the component of matrix itself [10]. In early OA the water content of the diseased cartilage increase and the cartilage swells, the collagen fibers are usually smaller and not tightly organized, the proteoglycan component of cartilage decrease markedly as disease progress with shortening of the glycosaminoglycan chain and impaired molecular aggregation [10].

Osteoarthritic cartilage is characterized by an increase in anabolic and catabolic activity, in the early stages the synthesis of collagen, proteoglycan, and hyaluronate is increased and chondrocyte tend to replicate at the same time the synthesis of degenerative enzyme such as collagenase, stromelysin, gelatinase, and hyaluronidase increased, the anabolic activity of chondrocyte become insufficient to keep up with the degenerative process, the final result is decreasing the ability of articular cartilage to withstand the required forces [10]. The reasons for the increased anabolic and catabolic activity of chondrocytes in OA are not well understood [33], mechanical forces may stimulate chondrocytes to produce a number of

inflammatory mediators that result in damage to the extra-cellular matrix including IL-1, IL-6, IL-8, IL-17, IL-18, monocyte-chemoattractant protein, nitric oxide, and reactive oxygen species [34].

It have been found that the role of free radicals in etiology of many disease include RA, OA, Alzheimer's disease, hypertension, and myocardial ischemia [35], These free radicals play a role in pathology of OA and RA via the effect upon lipids and cartilage, and the observational studies suggest that diets deficient in antioxidants may be associated with an increase OA or faster disease progression [36], numerous clinical studies testing the effectiveness of specific antioxidants or particular diets in the treatment of arthritis (OA, RA) through assessment serum level of malondialdehyde (MDA), Glutathione (GSH), superoxide dismutase (SOD), ceruloplasmin (CP), transferrin (TF), and catalase [37].

1.7 Diagnoses

Diagnosis of the patients with OA can be done depending on the following points

A. Patient history

It is important to identify significant past medical conditions or procedures and assess current acute and chronic medical conditions and symptoms [38,39]. Patients with OA are middle age or elderly and complain pain in the hand, knee, hip or foot joints, the pain worsens with the use of the affected joint and alleviated with rest, patients with OA may complain of instability or bucking especially when descending stairs or

stepping off curbs

B. Clinical manifestations

1. Signs and Symptoms

Physical sings in OA patients includes, bony enlargement of the affected joint, limitation the range of motion, crepitus on motion, malalignment and/or joint deformity, tenderness, erythema and unusual warmth [17,40]. Patient with OA complain a symptoms of joint pain, morning stiffness lasting less than 30 minute, joint swelling (mild or moderate), joint instability, and loss of joint function [40].

2. Radiographical features

Typical radio graphical findings in OA include narrowing of the joint space which result from the loss of cartilage, subchondral bone sclerosis and osteophyte (bony spurs), central erosions may be seen within the joint space, these erosion should be distinguishable from the periarticular erosions of RA [10,40].

3. Laboratory finding

Routine blood tests are normal in patients with OA includes erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), hemoglobin concentration (Hgb), and white blood cell counts (WBCs) [40].

In some patients the presence of an elevated ESR, high titer RF measurement or periarticular erosion changes may be the only way to distinguish RA from OA [10]. As shown in figure

(1.2).

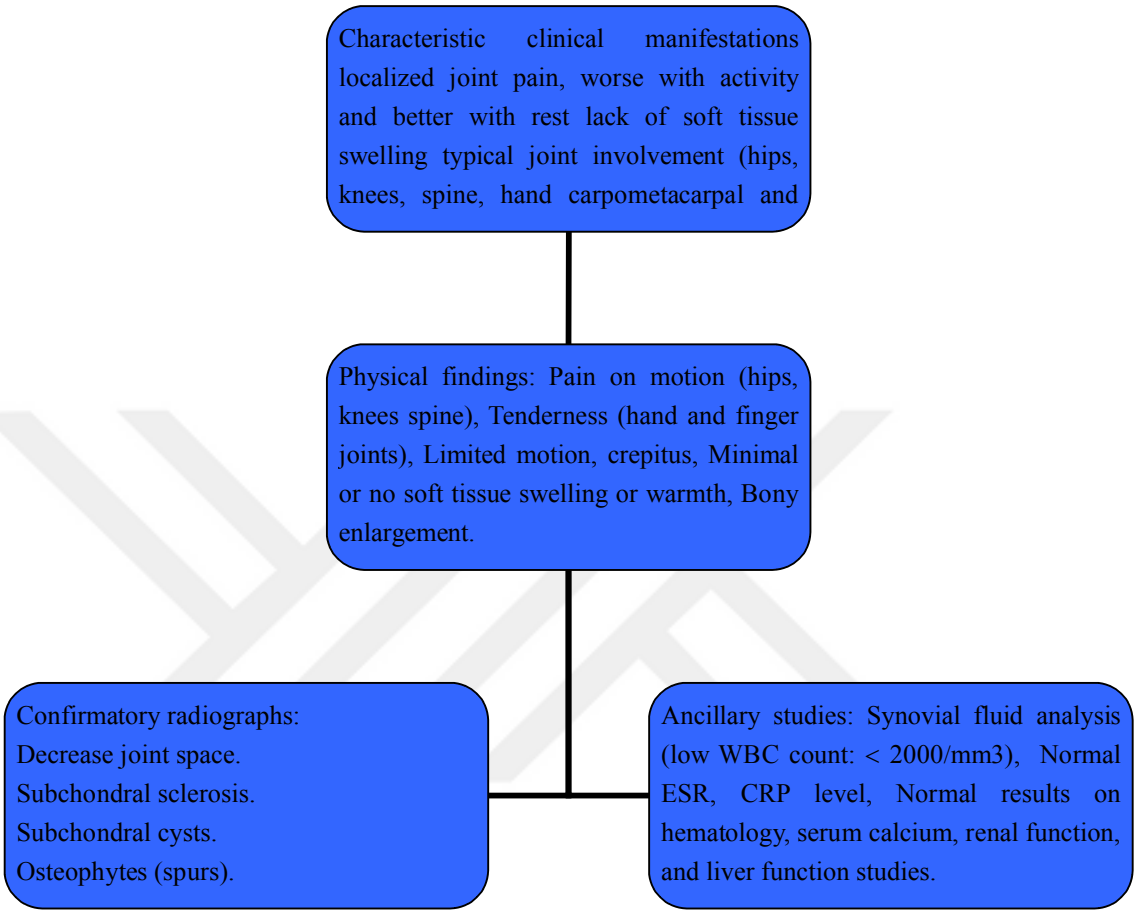


Figure (1.2): Diagnosis of Osteoarthritis ***(10)***

1.8 Differential diagnosis

Alternative diagnosis should be made in patients who are considered to be at low risk for OA (i.e. younger patients) or in those who have atypical pain patterns or atypical joint involvement. Patients with a relatively sudden onset of pain or with severe pain early in their conditions most often have sometimes other than OA. Crystal-induce arthritis should be always considered in patients with acute pain, particularly if swelling and erythema are prominent, calcium pyrophosphate deposition disease is common in the knees and hips and often coexists with OA, gout usually affects feet and ankle joints in early disease and is not often confused with OA thus examination of fluid from these joints for urate and crystals may be essential in differentiating gout from an inflammatory flare up of erosive OA, RA can usually be distinguished from OA on the basis of a different pattern of joint disease, more prominent morning stiffness and soft tissues swelling and warmth on physical examination [(10)].

1.9 Treatment

The treatment of OA is focused on relieving the signs and symptoms and improving the functions by preventing or retarding the degenerative biological process in articular

cartilage [41,42,43].

A. Non-pharmacologic treatment

Non-pharmacologic treatment have the potential to improve outcomes in **OA** patients include patient education, physical and occupational therapy, exercise, weight loss, and dietary measurement [44,45].

B. Pharmacologic treatment

The primary goal of the drug therapy in OA is to relieve pain and to reduce inflammatory action if present [46].

1. Acetaminophen

Up to 3000 to 4000 mg/day of acetaminophen in most cases can be given, and the doses should be limited in patients with exposure to other hepatotoxic substances [47].

2. Non-steroidal anti-inflammatory drugs (NSAIDs)

It is useful in treating OA patients mostly for their analgesic effect; in most patients they are more effective than acetaminophen. NSAIDs are associated with hypertension, fluid retention, renal compromise, gastric ulcers and bleeding therefore the strategies to reduce gastrointestinal tract (GIT) toxicity include the use of low doses of NSAIDs or concomitant use of misoprostol, H_2-Receptor antagonists, and proton pump inhibitors [48]. The use of selective cyclooxygenase-2 inhibitors such as (celecoxib, rofecoxib, and valdecoxib) posses less risk

of GI complications than nonselective cyclooxygenase inhibitors with same effectiveness in the treatment of OA, the later two COX-2 inhibitors (rofecoxib and valdecoxib) shows elevated risk for cardiovascular disease and have been withdrawn from market [(48)].

NSAIDs used to treat OA patients but it has destructive effect on the articular cartilage which lining the bones through suppressing proteoglycan synthesis by the chondrocyte thus inhibits cartilage synthesis and accelerate cartilage destruction, also aspirin inhibit the enzymes which are involved in the early stages of chondroitin sulfate biosynthesis thus accelerate destructive effects on articular cartilage [(16)]. Diclofenac sodium is anon steroidal anti-inflammatory drug taken to reduce inflammation and as analgesic reducing pain in condition such as arthritis or acute injury. It supplied as sodium or potassium salt and available as generic drug in a number of formulation used for minor aches pain and fever. The primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is through inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX), inhibition of COX also decrease prostaglandins in epithelium of the stomach making it more sensitive to corrosion by gastric acid (main side effect of diclofenac) chemical structure shown in figure (1.3). Diclofenac contraindicated in patients hypersensitive to diclofenac, 3rd trimester of pregnancy, stomach and duodenal ulcer, liver insufficiency and renal insufficiency [(49)].

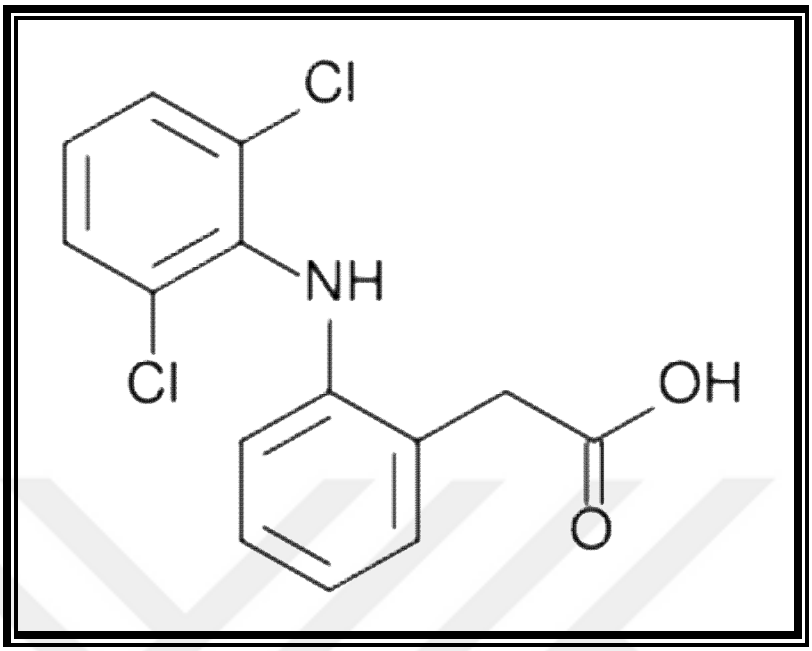


Figure (1.3): chemical structure for diclofenac ***(49)***

3. Steroids

Are the most effective anti-inflammatory agents, Intra-articular corticosteroid injections help for treating of the selected joints particularly during exacerbation which mentioned by increased pain and effusion, however they can also cause sever damage to chondrocytes in long-term use, in addition to the chronic use of strong steroids which leads to conditions that mimic Cushing's syndrome ***(16,50)***.

4. Opioids

Are generally avoided in OA patients but may be useful in some selected patients, used with caution particularly in elderly patients ***(48)***.

5. Tramadol

A centrally acting analgesic with dual mechanisms may give pain relief comparable to that achieved with acetaminophen

and codeine [48].

6. Topical capsaicin

May be useful in some patients especially those patients with knees and hands involvement [48].

7. Intra-articular hyaluronic acid derivatives

Given in a series of three to five injections weekly which have been shown to be superior to placebo in most studies and may be useful in relieving pain in selected patients with less advanced disease [51,52].

C. Dietary supplements (disease modification)

The major cause for OA is the destruction of chondrocytes as the logical approach to solving the problem by increasing substances in the joint that have chondroprotective and chondroregenerative effects, at the same time reducing inflammation which caused by the degenerative joint. These natural substances that have joint protecting and repairing properties include [53].

1. Glucosamine sulfate (GS)

Glucosamine is a simple molecule which composed of glucose and an amine, it stimulates the production of glycosaminoglycan (GAG) without glycosaminoglycan the collagen matrix will loose it is a gel-like nature and the ability to act as a shock absorbance [54,55].

2. Chondroitin sulfate (CS)

Chondroitin sulfate (CS) is aminopolysaccaride that contains a mixture of intact or partially hydrolyzed

glycosaminoglycan (GAG), it is a major structural component of cartilage and provides a matrix upon which collagen is built, it appears that taking GS and CS together offers significant improvement of OA patients than when they used separately due to addition advantage of glucosamine as structural building block of chondroitin and chondroitin stimulating effect on chondrocytes) [(56)].

3. Methyl sulfonylmethane (MSM)

It works by gives up its sulfur to form the collagen, keratin and essential amino acids (methionine, cysteine, and serum protein) which are essential components in maintaining normal body functions [(56)].

4. Bromelain

Bromelain refers to a group of sulfur-containing enzymes that digest protein, bromelain breaks down fibrin by stimulating the production of plasmin there by preventing fibrin from producing localized swelling (plasmin also block the formation of pro-inflammatory compounds) [(56,57)].

5. D-L phenylalanine (DLPA)

DLPA is an amino acid that is a primary building block for neural transmitters as well as pain control, it is slow the body's break down of endorphins as a result the body's internal painkiller will have a longer half-life and there for pain is

reduced. DLPA is an perfect agent for controlling inflammation and enhancing the effectiveness of analgesics. DLPA is often called nature's morphine because of its pain reducing effect [56].

6. Type I and type II collagen

Type I collagen is derived from chicken sternum cartilage, it contains the greatest numbers of anti-inflammatory and joints supporting proteoglycan including glucosamine sulfate. Type II collagen has the advantage of that it is much more absorbable when compared to ground cartilage, also decrease the enzymes attacks on the cartilage itself [16,56].

7. Sea cucumber

They are a source of whole food of chondroitin sulfate, it contains substances known as mucopolysacharides and chondroitins, sea cucumber significantly relieve joint pain without any side effects because it is a natural compound and work to rebuild the joint, it should be administered slowly and not immediately [16,56].

8. Essential fatty acids

Fish oil contains Omega 3 fatty acids and has been found to decrease inflammation that is associated with arthritis, fish oil works by decreasing the numbers of inflammatory messenger molecules which made by the body's immune system. High quality fish oil supplements are probably the best source of getting Omega 3 without the potential toxic metal effects [16,56].

9. S-adenosylmethione (SAMe)

It is a compound which made from the amino acid methione, it is effective in the relieving of pain in the arthritic joint as compared to NSAIDs [(56)].

10. Antioxidants vitamin E and C

A variety of reactive oxygen species (ROS) are formed continuously in tissues by endogenous and exogenous mechanisms, there is evidence that ROS may have a role in the pathogenesis of OA resulting from change cartilage synthesis and compromised cartilage repair, supplementation of antioxidants vitamin E, and C in high dose to patient with OA demonstrated a significant benefit, they appear to enhance the stability of sulfated proteoglycan in the collagen matrix.

Nutritional supplementations consideration:

Vitamin E: 400-800 IU/day, Vitamin C: 1000-3000 mg/day [(58)].

D. Alternative therapies [(59,60)].

1. Acupuncture

2. Low level laser therapy

3. Radiosynviorthesis

5. Surgery

1.10 Prognosis

OA is a slow progressive condition with variable prognosis [(18)], radiographically most joints will either remain stable or gradually worsen over a (5-15) year period, and progression of OA of the hand is particularly hard to measure because pain level frequently increase when the involved joints become

fused. The disease may progress more rapidly in the hips and knees of older women with osteopenic bone. The risk factors which might worsen the status include increased age and body mass index, properioceptive deficit, and pain intensity, whereas greater muscle strength, mental health self efficacy, social support, and aerobic exercise are associated with better outcomes [61].

1.11 Oxidative stress

Oxidative stress as a term used to describe the production of ROS from oxygen by oxidative reactions which are highly reactive molecules that damage the living organisms [62]. The ROS produced in body include [hydrogen peroxide (H_2O_2) [63,64], hypochlorous acid (HOCL) [65], superoxide radical ($O_2^{\cdot -}$) [66], hydroxyl radical ($^{\cdot}OH$) [67,68], peroxyl radical ($ROO^{\cdot}$) [69], singlet oxygen ($^1O_2$) [70], nitric oxide ($NO^{\cdot}$) [71], and peroxyl nitrite ($ONOO^-$) [69]]. Table (1.1) shows the effect of ROS during inflammation process [72].

Table (1.1): Oxidation and reactive oxygen species (ROS) during inflammation *(72)*

Factor	Effect
Oxidation	Modifies low-density lipoprotein. Inactivate α-1-protease inhibitor. Damage DNA. Cause lipid peroxidation.
ROS	Damage cartilage. Damage extracellular matrix. Inhibit collagen synthesis. Inhibit proteoglycan synthesis.

A. Free radicals (FRs)

Free radicals are atomic or molecular species with unpaired electrons in the outer (valence) shell of the molecule. Free radicals are activated in heated and rancid oil and by radiation in the atmosphere *(73)*.

B. Generation of free radicals

Free radicals can be generated both invivo and invitro by one of the following mechanisms [74].

- Homolytic cleavage of covalent bond.
- Loss of single electron from a normal molecule.
- Addition of an electron to a normal molecule [74].

C. Diagnosis of free radicals [75]

1. Electron spins resonance spectroscopy (ESR)

A widely used technique for studying free radicals and other paramagnetic species, alternatively referred to as "electron paramagnetic resonance" (EPR) it is related to nuclear magnetic resonance.

2. Chemical labeling

By quenching with free radicals, followed by spectroscopic methods like X-ray photoelectron spectroscopy (XPS) or absorption spectroscopy.

3. Use of free radical markers

Measurement specific or non-specific derivative of physiological subsances can be done by lipid peroxidation products, amino acid oxidation products (meta-tyrosin and ortho-tyrosin) and peptide oxidation product (oxidized Glutathione GSSG) [75].

4. Indirect method

Measurement of the decrease in the amount of antioxidants

e.g. reduced Glutathione (GSH) [75].

D. Lipid peroxidation

Lipid peroxidation mean the oxidation degradation of lipids, it is the process where by free radicals "steal" electron from the lipid in cell membrane that lead to cell damage, this process proceeds by a free radical chain reaction mechanism, a certain diagnostic tests are available for the quantification of the end products of lipid peroxidation specifically malondialdehyde (MDA) which is the most affects polyunsaturated fatty acids because they contain multiple double bonds in between which lie in methylene-CH_2-groups that possess especially reactive hydrogen, as with any radical reaction the reaction consist of three major steps which includes [76].

1. Initiation

Initiation is the step where by a fatty acid radical is produced, the initiators in the living cells most notably reactive oxygen species (ROS) such as

(· OH), which combines with a hydrogen atom to make water and a fatty acid radical [76].

2. Propagation

The fatty acids radical is not a very stable molecule so it react readily with molecular oxygen thereby creating aperoxyl-fatty acid radical, this high unstable species which reacts with

another free fatty acid producing a different fatty acid radical and a hydrogen peroxide or acyclic peroxide, and if it had reacted with itself, the cycle continues as the new fatty acid radical react in the same way [76].

3. Termination

The radical reacts it always produces another radical that is why the process is called "a chain reaction mechanism" the radical reaction stops when two radicals react and produce a non radical species, this happens only when the concentration of radical species is high enough for there to be a high chances of two radicals actually colliding [76], living organisms have evolved different molecules that speed up termination by catching free radicals and therefore protect the cell membrane, one important such antioxidant is alphatocopherol (vitamin E), and ascorbic acid (vitamin C) other antioxidants made within the body including the enzymes superoxide dismutase, catalase, and peroxidase [76]. As shown in figure (1.4).

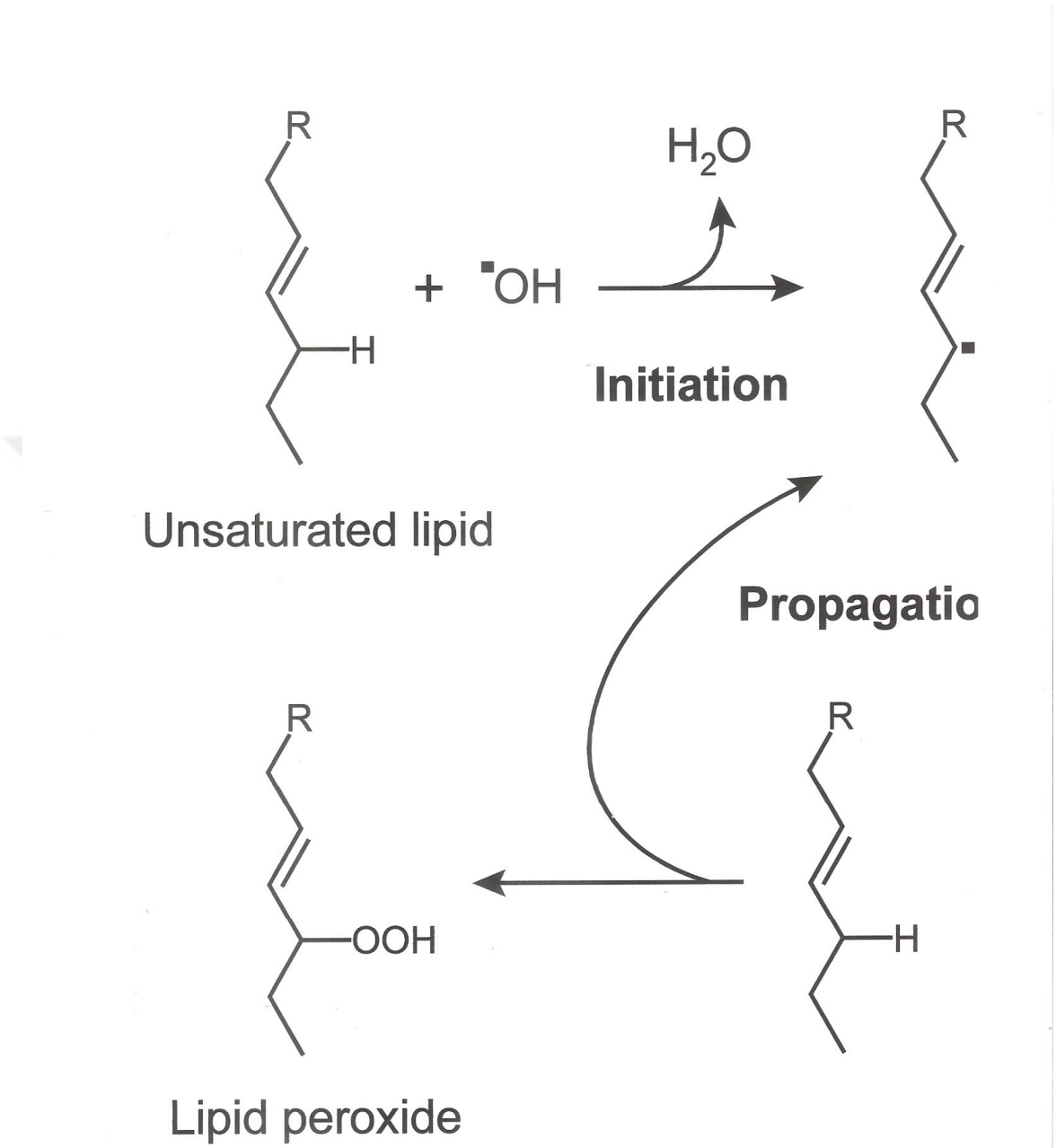


Figure (1.4): Mechanism of lipid peroxidation *(76)*

1.12 Antioxidants

A. Definition

Antioxidants are compounds exogenous or endogenous in nature which either prevent the generation of toxic oxidants or

intercept any generation and inactivate them by blocking the propagation of chain reaction which produced by these oxidants *(77)*.

B. Classifications

1. Enzymatic antioxidants

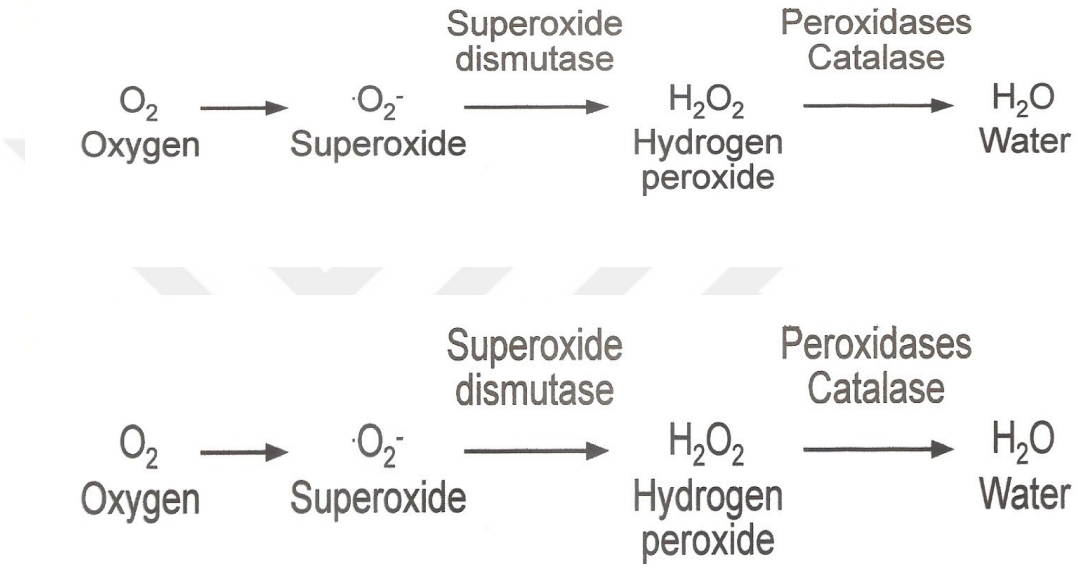


Figure (1.5): Enzymatic pathway for detoxification of reactive oxygen species *(79)*

2. Non-enzymatic antioxidants

Divided into two groups

- **Nutrient antioxidants**: Like beta-carotene, alphatocopherol, ascor-

  bic acid and bioflavonoid *(78)*.

- **Metabolic antioxidants**: Like glutathione, ceruloplasmin, albumin,

  bilirubin, ferritin, transferring, uric acid, and lactoferrin *(78)*.

1.13 Glutathione (GSH)

Is a non enzymatic metabolic antioxidant that protect cells from free radical toxins, is a tripeptide contains unusual peptide linkage between the amino group of cysteine and carboxyl group of the glutamate side chain. Glutathione reduces any disulfide bonds which form within the cytoplasmic proteins to cysteine's by acting as an electron donor. Glutathione is found exclusively in its reduced form, since the enzyme that reverts it from its oxidized form (GSSG) glutathione reductase is constitutively active and inducible upon oxidative stress, in fact the ratio of reduced glutathione to oxidized glutathione within the cells is often used scientifically as a measure of cellular toxicity [(80)], glutathione is synthesized in two adenosine triphosphate-dependent steps.

First: Gamaglutamyl cysteine is synthesized from L-glutamate cys-

teine via the enzyme gammaglutamyl cysteine synthetase, this

reaction is the rate limiting step in the glutathione synthesis.

Second: Glycine is added to the C-terminal of gammaglutamyl

cysteine via the enzyme glutathione syntheses [(81)].

1.14 Vitamin E

It is fat soluble vitamin with antioxidant properties that protect cell membranes from oxidation by reacting with lipid radicals which are produced in the lipid peroxidation chain

reaction [82]. Vitamin E is a collective name for a set of eight related tocopherols and tocotrionols [83,84], include alpha, beta, gamma, and delta tocopherol and alpha-beta-gamma, delta tocotrienols, D-alpha tocopherol account for 80% of the activity of the vitamin [84,85], in theory when a vitamin E supplement is labeled "400 IU" it should have the same level of activity regardless of its source, this is purportedly achieved by using more synthetic vitamin E to reach the same potency as a lesser amount of natural vitamin E, for example 100 IU of vitamin E requires about 67 mg of the natural form but closer to 100 mg of the synthetic, natural vitamin E may be as much as twice as bioavailability as synthetic vitamin E (1.36 times as is generally accepted). High level of vitamin E can be found in the following foods, Wheat germ, corns, nuts, seeds, olives, spinach and green leafy vegetable (asparagus and vegetable oils) [86]. Figure (1.6) show vitamin E structure. Note 1 IU = 1/40th (0.025) µg [85].

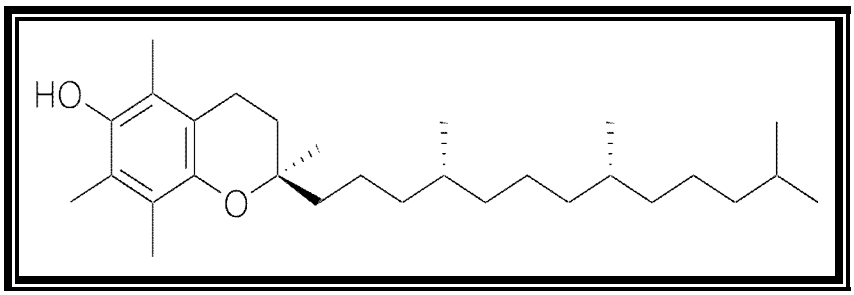


Figure (1.6): α-tocopherol (vitamin E) chemical structure [86]

- ***Absorption***

The absorption of tocopherols and tocotrionols will not be complete without the presence of dietary fat; therefore their absorption in blood stream will be erratic especially when taken in fasting condition [85].

- ***Mechanisms of action***

Vitamin E act to prevent the peroxidation of membrane phospholipids through its antioxidant action, tocopherol-OH can transfer a hydrogen atom with a single electron to a free radical before it can interact with cell membrane proteins or generate lipid peroxidation, when tocopherol-OH combines with the free radicals, it becomes tocopherol-O·, which combined with other free radical to form LOO-alpha tocopherol as follow [87].

Alpha tocopherol + LOO· ⟶ Alpha tocopherol· +LOOH

Alpha tocopherol· + LOO· ⟶ LOO-alpha tocopherol [87].

Also vitamin E has been shown to modulate the activity of cyclooxygenase and lipoxygenase enzymes, which are involved in the conversion of arachidonic acid to pro-inflammatory prostaglandins and leukotrienes. Experimental evidence demonstrates that vitamin E and other antioxidants reduce the synthesis of pro-inflammatory [87].

- ***Therapeutic effects***

Vitamin E decreases the incidence of ischemic heart

disease, cataract, osteoarthritis, and rheumatoid arthritis [88,89].

1.15 Vitamin C

Water soluble antioxidant present in citrus fruits, tomatoes, and green leafy vegetable. Human unable to synthesize L-ascorbic acid from d-glucose due to absence of the enzyme L-glucolacton oxidase, therefore human should obtain ascorbic acid from dietary sources [90]. Vitamin C deficiency cause scurvy in the humans, it is also widely used as a food additives [91,92]. Chemical structure of vitamin C shows in figure (1.7).

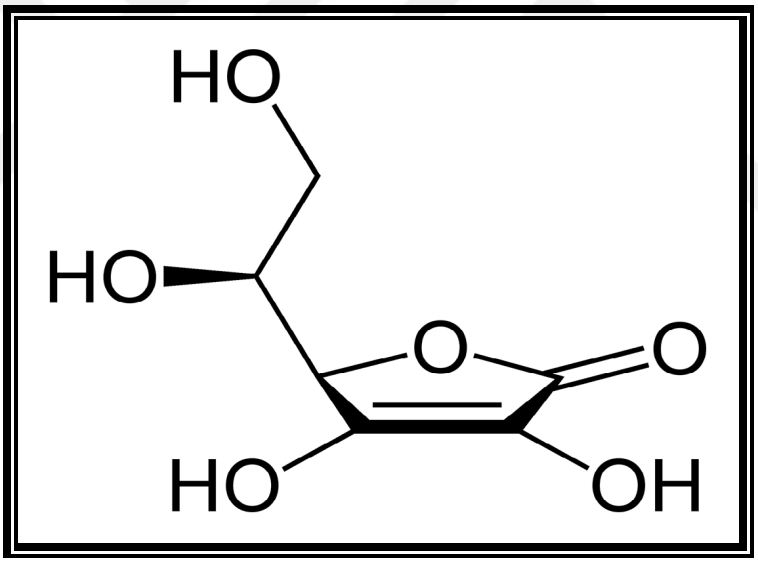


Figure (1.7): Ascorbic acid (vitamin C) chemical structure [93]

- ***Absorption***

In human and guinea pigs, the absorption of vitamin C occurred in the buccal mucosa, stomach, and small intestine. The buccal absorption is mediated by passive diffusion through the membrane of the buccal cavity, while gastrointestinal

absorption is through an efficient and an active sodium dependent energy requiring and carrier-mediated transport mechanism then re-absorption occurs in the kidneys. Since the absorption mechanism in the gut and the kidney can reach a saturation point, its better to take multiple and smaller doses of vitamin C throughout the day than one large dose [(94)].

- ***Biological significance***

Regarding biological significance vitamin C has been associated with the slowing of (OA) progression in animal and human studies [(95)].

- ***Mechanisms of action***

It is water soluble chain breaking antioxidant act as scavenger to free radicals and reactive oxygen species which are produced during metabolic pathway, vitamin C has the ability to act as a reducing agent (electron donor) react rabidly with O_2^- and e^- with $^{\cdot}OH$ to give DHA, which itself can act as a source of vitamin C as follow:

Ascorbic acid + $2O_2^{\cdot -}$ + $2H^+ \longrightarrow$ H_2O_2 + DHA [(96)].

Vitamin C act as an electron donor for eight different enzymes [(97)]:

- Three participate in collagen hydroxylation, these reaction will add

hydroxyl groups to amino acid proline or lysine in the collagen

molecule [98,99].

- Two are necessary for synthesis of carnitine which is essential for

the transport of fatty acid in mitochondria for ATP generation [100].

- Dopamine beta hydroxylase participate in the biosynthesis of

nor epinephrine from dopamine [101].

- Another enzyme adds amide groups to peptide hormones, greatly

increasing their stability, and one modulate tyrosine metabolism [102,103].

- ***Toxicity***

Toxicity normally does not occur but high levels of vitamin C interfere with copper absorption, and vitamin C should be avoided by those patients who suffer from kidney stones [104].

1.16 Investigation parameters

The laboratory investigation parameters are important for making definitive diagnosis in addition to patient history, physical examination and radiographic evaluation of the disease [105].

A. Sedimentation rate (ESR)

It is highly sensitive blood test, non specific, when inflammation is present in the body certain proteins cause red blood cells to stick together and fall more quickly than normal to the bottom of the tube these proteins are produced by the liver and the immune system under many abnormal conditions such as infection, autoimmune disease or cancer, this blood test is done to find out if inflammation present, to cheek the progress of the disease, and to see how well the treatment is working [(106)]. The ESR is often elevated in RA, SLE, vasculitis disorder or gout [(105)].

B. C-reactive protein (CRP)

It is a blood test that measures the amount of protein called C-reactive protein in the blood. Normal values may vary between laboratories; normally it is less than 10 mg /L (SI units). Parameters which affects CRP normal range include increase in age, body mass index, systolic blood pressure, plasma level of proinflammatory cytokine IL-6, hematocrit, viscosity, WBCs count, coagulation factors [fibrinogen, F(VII), F(VIII), F(IX)], and women on oral contraceptive [(107,108)]. CRP level can be used to see the response to the treatment which will rise quickly and then return to normal if the treatment is working [(109)].

C. Rheumatoid factor (RF)

The rheumatoid factor is an Ig antibody directed against the Fc portion of IgG, it is non specific factor present in the serum of 70%-90% of patients with RA, it has low true sensitivity and variable specificity among patients with a variety of infectious

or inflammatory disorders, including viral infection, sub-acute bacterial endocarditis, and hepatitis. False-positive results are common, healthy patients noted to have appositive RF occasionally develop RA over time but most do not, it is documented to be 5% among population, IgM rheumatoid factor is most commonly detected IgG and less frequently IgA , presence of IgG rheumatoid factor is associated with higher rate of bone erosion and systemic complications [(105)].

D. Malondialdehyde (MDA)

It is a product of lipid peroxidation and thereby functions as a marker of oxidative stress, the level of MDA in plasma or serum has been reported to be higher in RA and OA patients than in control subjects, recent study have reported a correlation between ESR and MDA plus another lipid peroxidation product, so MDA test used to indicate the severity of lipid peroxidation which occur due to reactive oxygen species that causing damage to the lipids [(110,111)].

E. Hemoglobin concentration (Hgb)

Hgb is the oxygen carrying compound containing in the RBCs, therefore the total Hgb concentration primarily depends on the number of red cells in the blood sample. Hemoglobin (Hgb) is an organic molecule composed of protein, lipid, minerals, and vitamins, it has two primary function, gas transport (oxygen and carbon dioxide) and as a buffer to maintain the body's acid base balance, most of Hgb molecules within an erythrocyte are physiologic Hgb, it is capable of transporting the gases required by the body. Oxyhemoglobin is

formed by the reversible oxygenation of iron which results in collapsing of the molecule around the oxygen holding it in place until the RBC reach its target cell when the oxygen is released deoxyhemoglobin is formed.

Non-physiological hemoglobin (methemoglobin, sulfhemoglobin, and carboxyhemoglobin) also exist, these forms of Hgb are incapable of transporting oxygen and when present in excess can cause serious and even fatal disease [(112)]. Hgb content of the whole blood is determined by photometric method and recorded as gm/dL (normal range: male 16.0 ± 2.0, female 14 ± 2.0) [(113)].

Anemia can be indicated through following results of blood study.

1. Low Hgb level (male <12gm/dl, female <10gm/dl)

2. Low hematocrite level (male <47ml/dl, female <47ml/dl)

3. Low serum ferretin or iron level.

4. Low RBC count [(114)].

Hgb values are affected by age, sex, pregnancy, diseases [(115)].

Hemoglobin (gm/dl) = hematocrite (% PCV) x 0.34

Hemoglobin (gm/dl) = hematocrite (decimal fraction) x 34 [(116)]

F. White blood cells (WBCs)

Unlike RBCs, perform no physiological function within the vascular system, the blood serves as a transportation network that allows WBCs to move from their site of origin (bone marrow) into various body tissues and cavities, Neutrophils are

the most abundant of the circulating WBCs followed in order of frequency by Lymphocytes, Monocytes, Eosinophils, and basophiles [(113)]. Normally count (3.2-9.8 cell x10^9/L).

G. Glutathione test (GSH)

Glutathione is a tripeptide antioxidant protects cells from free radicals by acting as an electron donor, it is found in its reduced form (GSH) since enzyme that reverts it from its oxidized form (GSSG), glutathione reductase is constitutively active and inducible upon oxidative stress [(117)]. Measurement of serum concentration (GSH) indicate the progression of lipid peroxidation process, the negative correlation between (GSH) serum concentration and oxidative stress used to assess the response to antioxidants therapy given to patients as shown in figure (1.8) [(118)].

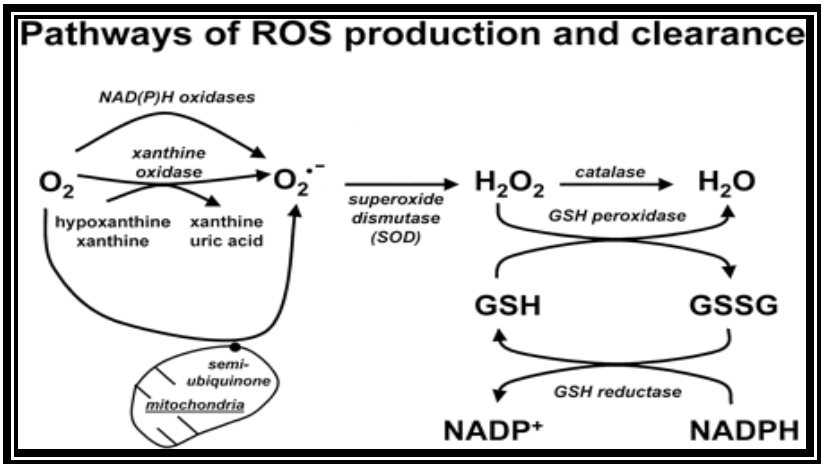


Figure (1.8): Action of (GSH) as an electron donor to free radicals [118]

1.17 Osteoarthritis outcome score

It is useful, reliable, valid and responsive instrument for assessment of patients' outcomes especially in elderly subjects with advanced OA [119]. OA outcome score has high test-retest reproducibility; its validation work is ongoing and is currently being in several clinical studies involving patients with meniscus injury, cartilage injury, and osteoarthritis.

Pain is defined as unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in term of such damage [120]. It is complex biological, psychological, and social process and significant factor that influences function and quality of life for individuals with arthritis, pain with movement is the principle symptoms of OA patients, although cartilage tissues contain no pain receptors, sensation of pain likely results from inflammatory mediators, bone damage and mechanoreceptors in the surrounding joint, the pain usually has an insidious onset and tends to increase with disease duration, also pain at rest at night may accompany advanced disease [121].

Aims of this study

This study was designed to evaluate the effect of antioxidants (vitamin E and vitamin C) as an anti-inflammatory in reducing pain and joint disability in OA patients treated with diclofenac sodium in a single or combined form through using, symptoms and pain score and laboratory tests (MDA and GSH).

2.Patients and Methods

2.1 Materials

2.1.1 Diagnostic Kits used in this study

The Kits used in this study are mentioned in table (2.1) with their manufacture and origin.

Table (2.1): Kits used in this study

Kit	Manufacture	Origin
CRP latex kit (100 test)	SPINREACT	Spain
RF latex kit (100 test)	SPINREACT	Spain

2.1.2 Instruments used in this study

The instruments used in this study are mentioned in table (2.2) with their manufacture and origin.

Table (2.2): Instruments used in this study

Instrument	Manufacture	Origin
Eppendorf centrifuge 5417 R	AG 22331-Hamburg	Germany
Table top low speed centrifuge	Triup international CORP	USA
UV-Visible spectrophotometer	Cary 100 conc.	USA
Water bath electro-thermal	CAT No. BG 7311	England
Electronic sensitive balance	Precisa XT 220A	Switzerland
Digital sensitive balance	Diamond model 250	France
Micropipette	SALMED	Germany
pH meter	HANNA instruments	Korea

2.1.3 Chemicals used in this study

The chemicals used in this study are mentioned in table (2.3) with their manufacture and origin.

Table (2.3): Chemicals used in this study

Chemical	Manufacture	Origin
Potassium di hydrogen phosphate (white crystal powder) $\mathbf{KH_2PO_4}$	RIEDAL-DE-HAEN AG	Germany

Disodium hydrogen phosphate (white crystal powder) **Na_2HPO_4**	RIEDAL-DE-HAEN AG	Germany
Di thionitroso benzoic acid (**DTNB**) (yellow powder)	FLUKA	England
Sulfa salicylic acid (**SSA**) (white crystal powder)	Himedia	Germany
Tri chloro acetic acid (**TCA**) (hygroscopic white crystal)	Merck	Germany
Thiobarbituric acid (**TBA**) (yellow powder)	BDH	Poole, England
Concentrated HCl (37%) (solution)	Merck	Germany

2.1.4 Drugs used in this study

The drugs used in this study are mentioned in table (2.4) with their potency, doses, manufacture, and origin.

Table (2.4): Drugs used in this study

Drug	Potency	Dose	Manufacture	Origin
Diclofenac sod. Retard film coated Tab.	100 mg	100 mg/day	Olfen	Swıss
Vitamin E soft- gelatin Cap. (α-tocopheryl acetate USP)	400 mg	800 mg/day	Puritan's Pride	Usa
Vitamin C Tab. (ascorbic acid)	500 mg	1000 mg/day	Natural Factors Vitamin C	Canada

2.2 Subjects

2.2.1 Patient selection

Under clinical observation for the patients was carried out over nine months from August 2013 till May 2014 in the hospital.

sixty one patients (52 female and 9 male) with OA and eleven apparently healthy control subjects (7 female and 4 male) were participated in this study, the sixty two patients were diagnosed to have OA by specialist doctor depending on clinical manifestation (signs and symptoms), laboratory investigation,

and X–ray and all that done after getting the permeation from them according to the law and the agreement to follow the progress in their conditions.

2.2.2 Patients exclusion criteria

1. Patients hypersensitive to vitamin E, vitamin C or diclofenac sod.

2. Patients have secondary OA due to injury or trauma.

3. Patients with history of peptic ulcer, gastric ulcer and asthma.

4. Patients on corticosteroids, disease modifying anti Rheumatoid drugs

(DMARDs) or other locally injected (in joints) therapy.

2.2.3 Data collection

Patient information sheet figure (2.1) include all details which were recorded for patients who are clinically diagnosed to have osteoarthritis in addition to apparently healthy control subjects such as name, age, sex, weight, height, address, occupation, clinical manifestations (signs and symptoms) before treatment and 2**,** 4**,** 6**,** and 8 weeks after treatment , X–ray of the affected joints, laboratory findings include erythrocyte sedimentation rate ESR mm/hr, serum C-reactive protein CRP mg/L, Rheumatoid factor RF IU/mL, Hemoglobin concentration Hgb gm/dL, serum malondialdehyde MDA μmol/L, serum Glutathione GSH μmol/L, and white blood cells count WBCs No. x 10^9/L were measured before and every 2**,** 4**,** 6**,** and 8

weeks after treatment with past medical history PMHx, family history FHx, and current patient medications were documented.

Wt = Kg H = cm

- Serial No. :

- Name:

- Age: Sex:

- Occupation:

- Address:

History of present illness:

''Diagnosis as osteoarthritis''

1. Clinical observation (signs and symptoms):

Before treatment:

2 weeks after treatment:

4 weeks after treatment:

6 weeks after treatment:

8 weeks after treatment:

2. X–ray for affected join(s):

3. Laboratory test:

Duration of treatment	ESR mm/hr	CRP mg/L	RF IU/mL	Hgb gm/dL	GSH µmol/L	MDA µmol/L	WBCs No.x10^9/L
Before treatment							
2 weeks after Tx.							
4 weeks after Tx.							
6 weeks after Tx.							
8 weeks after Tx.							

- Treatment:

- Notes:

Figure (2.1): Patient's information sheet

2.2.4 Blood sample collection

Venous blood 10 mL was obtained from forearm of each patient by vein puncture at abase line (before initiation of therapy), and 2**,** 4**,** 6**,** and 8 weeks after treatment, the blood samples were transferred from sterile 10 cc syringe, three mL of blood transferred to 5 mL potassium EDTA tube that used for ESR, WBCs, and Hgb concentration measurement and the remained 7 mL of blood transferred to plane tube (EDTA free tube) and left to be coagulated and then centrifuge for 15 mins. at 3000 rpm. and 2 mL of serum was used for GSH and MDA tests while remaining serum was stored in a deep freezing (–20 C^0) to be used later for CRP and RF latex agglutination tests.

2.2.5 Age distribution

Sixty one patients clinically diagnosed to have osteoarthritis were included in this study with age range (39-75) years, and mean age ± SD was (52.6 ± 8.9) years as mentioned in table (2.5).

Eleven apparently healthy control subjects were included in this study with age range (38-60) years and mean age ± SD was (51 ± 7.3) years as mentioned in table (2.5).

Table (2.5): Age distribution for OA patients and apparently healthy control subjects participated in this study

Age interval (years)	Numbers of patients	Number of subjects
(30-39)	**1**	**1**
(40-49)	**17**	**4**

(50-59)	**25**	**4**
(60-69)	**14**	**2**
(70-79)	**4**	**-**

2.2.6 Weight distribution

The sixty one OA patients were included in this study have the weight range of (45-133) Kg, and weight mean ± SD was (83.5 ± 14.7) Kg as mentioned in table (2.6) as a body mass index parameter.

Table (2.6): Weight distribution for OA patients and apparently healthy control subjects [(124)].

Body mass index (BMI)	Female	Male	Number of (OA) patients	Number of healthy control subjects
Under weight	**< 19.1**	**< 20.7**	**1 female**	**Zero**
Normal range	**(19.1-15.8)**	**(20.7-26.4)**	**6 female**	**6 female + 3 male**
Marginally over weight	**(25.8-27.3)**	**(26.4-27.8)**	**7 female + 2 male**	**2 female + 1 male**
Overweight	**(27.3-32.3)**	**(27.8-31.1)**	**16 female + 5 male**	

Overweight obese	**>32.3**	**>31.1**	**22 female + 2 male**	

The standard definition of body mass index (BMI) is affected by many factors including, age, sex, race/ethnicity, and nationality affect body composition. The muscular people, athlete, and body builders have high BMI values but are not fat *(125)*.

2.2.7 Patients groups

OA patients who enrolled in this study were diagnosed by specialist as having osteoarthritis while attending Baghdad teaching hospital, and eleven apparently healthy control subjects 7 female and 4 male patients were divided into five groups according to the treatment given as follow:

Group (A): Eight patients [(6) female, and (2) male] with osteoarthritis

treated with 100 mg diclofenac sod. once daily for eight weeks.

Group (B): Twenty patients [(18) female, and (2) male] with osteoarthritis

treated with 100 mg diclofenac sod. once daily plus vitamin E

400 mg twice daily for eight weeks.

Group (C): Nigh teen patients [(17) female, and (2) male] with osteoarthritis

treated with 100 mg diclofenac sod. once daily plus vitamin C

500 mg twice daily for eight weeks.

Group (D): Fourteen patients [(11) female, and (3) male] with osteoarthritis

treated with 100 mg diclofenac sod. once daily plus vitamin E

400 mg twice daily plus vitamin C 500 mg twice daily for eight

weeks.

Group (E): Eleven [(7) female, and (4) male] apparently healthy control

subject used to standardized normal laboratory values for normal

population.

The numbers of subjects in each group with their antioxidants treatment are shown in figure (2.2).

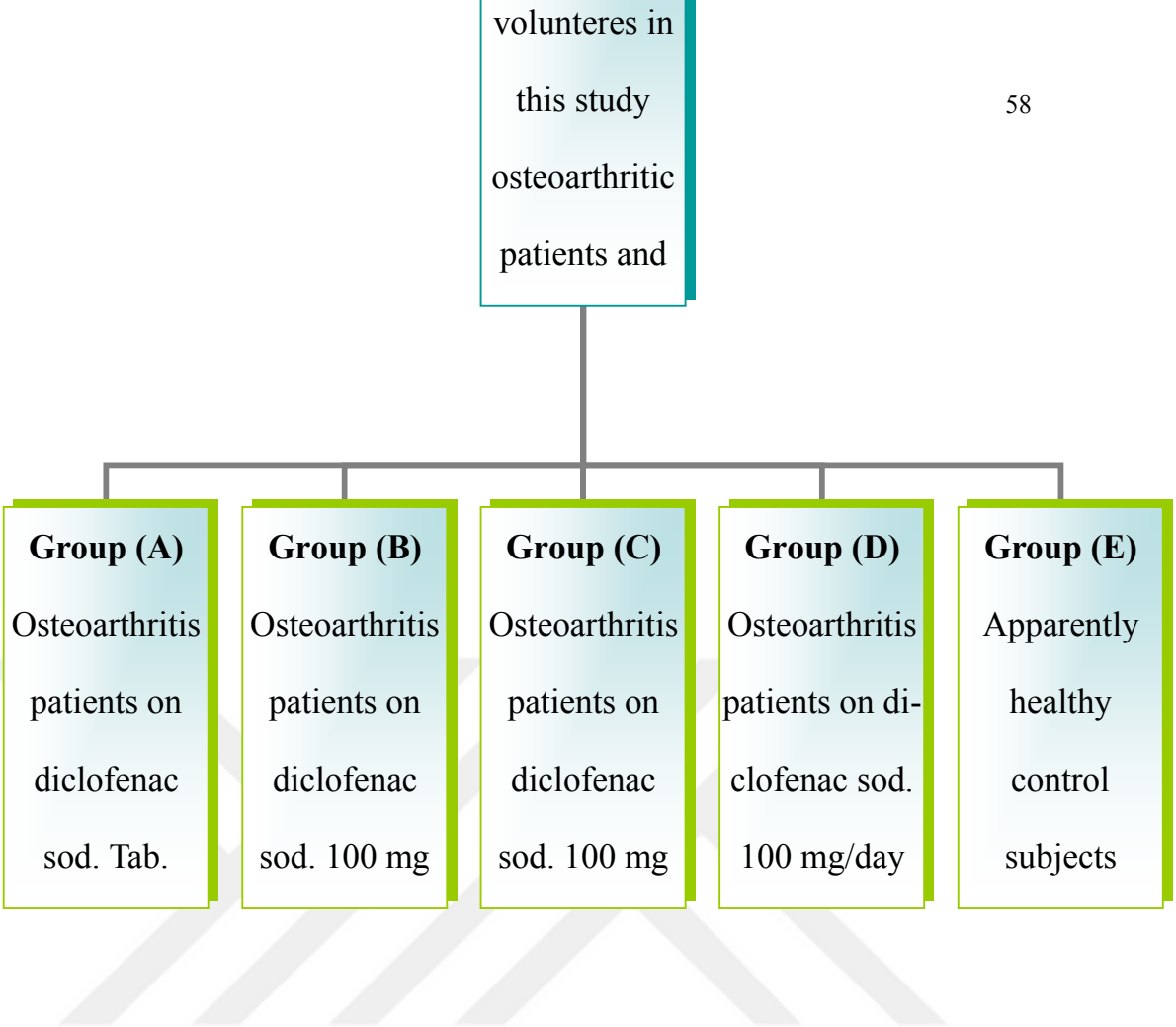


Figure (2.2): Schematic diagram show the number of volunteers in each group with there therapeutic regimen

2.3 Laboratory analysis methods

2.3.1 Measurment Erythrocyte sedimentation rate (ESR) method

The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocyte (zero potential), when inflammatory process is present the high proportion of fibrinogen in the blood cause red blood cells to stick to each other, the red cells form stacks called "rouleaux" which settle faster, rouleaux formation can also occur in association with some lymphproliferative disorders in which one or more immunoglobulin are secreted in high amount, the ESR test can be perform using anticoagulant blood that placed in an upright tube known as a Westergren tube and the rate at which the RBCs fall is measured and reported in mm/hr [(126)].

- The ESR increase by any cause or focus of inflammation.
- The ESR decrease in sickle cell anemia, polyerythemia, and

congestive heart failure the usually test termed as Westergren test [(127)].

The rule for calculating normal maximum ESR values in adult is given by following formula.

$$\text{ESR } (mm/hr) \leq \frac{25\ (in\ years) + 10\ (if\ female)}{2}$$

Upper normal limit of ESR for males less than 50 years of age is 15 mm/hr and for females less than 50 years of age is 20 mm/hr, males and females who are over 50 years of age using previous formula to obtain normal upper limit [126,127].

2.3.2 Measurement C-reactive protein (CRP) method

It is acute phase protein which reflect a measure of the acute-phase response, the term " acute phase" refers to local and systemic events that accompany inflammation, local responses include: vasodilatation, platelet aggregation, neutrophil chemotaxis, and release of lysosomal enzymes, systemic responses include: fever, leukocytosis, and a change in hepatic synthesis of acute phase proteins [128]. The reagents used in this test are mentioned in table (2.7).

Table (2.7): Reagents used for (CRP) particle agglutination test

Reagents	composition
Latex	latex particles coated with goat IgG anti-human (CRP)
Control +ve	human serum with a (CRP) concentration > 20 mg/L
Control –ve	animal serum

Procedures:

- Allow the reagent (latex) and sample (serum) to reach room temperature.
- Place 50 μL (one drop) of the sample and 50 μL (one drop) of the CRP latex reagent into circle on the slide test.
- Mix the drops with a stirrer, spreading them over the entire surface of the circle.
- The presence of agglutination indicate a CRP positive patient and absence of agglutination indicate a CRP negative patient.

The approximate CRP serum concentration can be measured using the titration method as follow:

- Make serial two fold dilution of the sample using 9 g/L saline solution.
- Proceed for each dilution as in the qualitative method (previous procedure).

The approximate CRP serum concentration in the patient sample is calculated as follow:

6 x CRP titer = mg/L [approximate (CRP) serum concentration]

The CRP and ESR regarded as marker of choice in monitoring the acute phase because.

1. Have relatively short lag-time from the moment of stimulus.

2. Cost effective.

3. They increase in concentration relatively high compared to basal concentration [(128)].

2.3.3 Measurement Rheumatoid factor (RF) method

This was considered as an earliest, widely used method; clinically rely on the agglutination properties of the IgM class of RF, the presence of RF is detected by agglutination or flocculation of the respective indicator system. Semi-quantitative analysis to determine the antibody content of a serum involving doubling dilutions of the serum and determination of an end point (the last doubling dilution at which agglutination can be visualized), the reciprocal of this dilution is known as "antibody titer" [(128)]. The reagents used in this test are mentioned in table (2.8).

Table (2.8): Reagents used for (RF) particle agglutination

test

Reagents	composition
Latex	Latex particle coated with human gamma-globulin
Control +ve	Human serum with a (RF) concentration > 30 IU/mL
Control –ve	Animal serum

Procedures:

- Allow the reagent (latex) and sample (serum) to reach room temperature.
- Place 50 μL (one drop) of the sample and 50 μL (one drop) of the RF

  latex reagent into circle on slide test.
- Mix the drops with a stirrer, spreading them over the entire surface of the

  circle.
- The presence of agglutination indicate a RF positive patient and absence

  of agglutination indicate a RF negative patient.

The approximate RF serum concentration can be measured using the titration method as follow:

- Make serial two fold dilution of the sample using 9 g/L saline solution.
- Proceed for each dilution as in the qualitative method (previous procedure).

The approximate RF serum concentration in the patient

sample is calculated as follow:

8 x RF titer = IU/mL [approximate (RF) serum concentration]

2.3.4 Measurement serum malondialdehyde concentration method (MDA)

Malondialdehyde MDA is the end product of lipid peroxidation was analyzed according to the Buege, and Aust method (1978), based on the reaction of MDA with thiobarbituric acid TBA to form a red chromophore, which can be quantitated spectrophotometrically.

Reagents:

- 15 gm of trichloroacetic acid TCA.
- 0.375 gm of thiobarbituric acid TBA.
- Dissolve 1 and 2 in (0.25 N) HCl complete volume to 100 mL.

Procedures:

- 0.5 mL of serum is added to 1 mL of TBA reagent [15 gm trichloro-

  acetic acid, and 0.375 gm thiobarbituric acid, in 100 mL of (0.25 N)

  HCl], the content is mixed well and heated in water bath using boiling

  water for (15-30) minutes then cooled.
- After cooling the precipitate was removed by centrifugation at 3000 rpm

  for 15 minutes.
- The absorbency determined at 535 nm against reagent blank which was

containing all the reagents minus the serum is as follow.

MDA (µmol/L) = (ΔA/1.56) x 10

Where (**ΔA**) is the difference in absorbance between sample and blank (absorbance of sample – absorbance of blank).

2.3.5 Measurement serum glutathione concentration method (GSH)

Glutathione exists in reduced form GSH and oxidized form GSSG, in reduced form the thiol group of cystiene is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules such as reactive oxygen species, in donating an electron, glutathione itself becomes reactive but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG), such reaction is possible due to the relatively high concentration of glutathione in cells, GSH can be regenerated from GSSG by the enzyme glutathione reductase.

Reagents:

- 4 gm of sulfa-salicylic acid SSA in distilled water to make 100 mL of

  4% sulfasalicylic acid solution SSA.
- 9.073 gm of potassium dihydrogen phosphate KH_2PO_4 in distilled

  water to make 1000 mL of KH_2PO_4 solution A acid.
- 11.87 gm of disodium hydrogen phosphate Na_2HPO_4 in distilled water

  to make 1000 mL of Na_2HPO_4 solution B base.
- 0.0346 gm of dithionitroso benzoic acid DTNB added to 100 mL of

buffer phosphate pH = 8 freshly prepared by adding 3.7 mL of solution

A and complete volume to 100 mL by solution B.

Procedures:

- 0.5 mL of serum added to 0.5 mL of 4% salfasalicylic acid solution

(SSA), mixed well and then centrifuge.

- Take 0.5 mL of supernatant by micropipette and added to 4.5 mL of

freshly prepared DTNB solution .

- Mixed well and leave it for 5 minute then read absorbency at 412 nm.
- Calculate serum concentration of glutathione using following equation.

$$\mathbf{C_0\ (\mu mol/L) = (A/E)}$$

Where is $\mathbf{C_0}$ = Original concentration (serum glutathione concentration) in

μmol/L.

A = Absorbency at 412 nm.

E = Extriction coefficient equal 13600 M^{-1} cm^{-1}.

2.3.6 Measurement hemoglobin concentration method (Hgb)

Hemoglobin in the body present as a mixture of oxy-hemoglobin, carboxyhemoglobin, met-hemoglobin, and sulphahemoglobin, each of these has certain color so for

estimation of hemoglobin concentration we must convert all of these to one standard form which is acid hematin. The intensity of color is measured by comparing it with standard solution; the results are expressed as % from normal or as gm/mL of blood.

Male: (14–18) gm/dL

Female: (12–16) gm/dL

Procedures:

- Put (0.1 N) HCl in graduated tube to the mark 10.
- Take blood in the hemoglobin pipette to the mark 20.
- Expel the blood under the acid and mix them by sucking and expelling many time.
- Wait 10 minutes then dilute with distilled water drop by drop and the color compared with that of the standard solution to reach acceptable color of the standard solution.

2.3.7 White blood cells count (WBCs)

The principle of white blood cells count depend on accurate dilution of measured blood. WBC count required ahemacytometer, microscope, and special pipette for diluting blood.

Procedures:

1. Place the pipette tip into the blood sample.

2. Draw blood up in the tube to the 0.5 mark.

3. Immerse the pipette tip in 2% v/v acetic acid and then fill it by this fluid to

11 mark.

4. The counting chamber (hemacytometer) is conveniently located on the

microscope stage.

5. Shake pipette to insure adequate mixing and expel the fluid from the

pipette stem and wip the tip.

6. Place the tip of the pipette exactly at the junction of the cover glass

between the cover glass and the champer.

7. The capillarity of the space between the cover glass and the champer will

immediately pull fluid from the pipette to fill the champer.

8. The WBC counted in the four large out side squares (each squares have

1mm^2 area so total area counted 4mm^2).

9. Cells touching the upper and left hand boundary line of the main squares

are counted while lower and right hand boundary lines are not counted

(count at 100 X magnification).

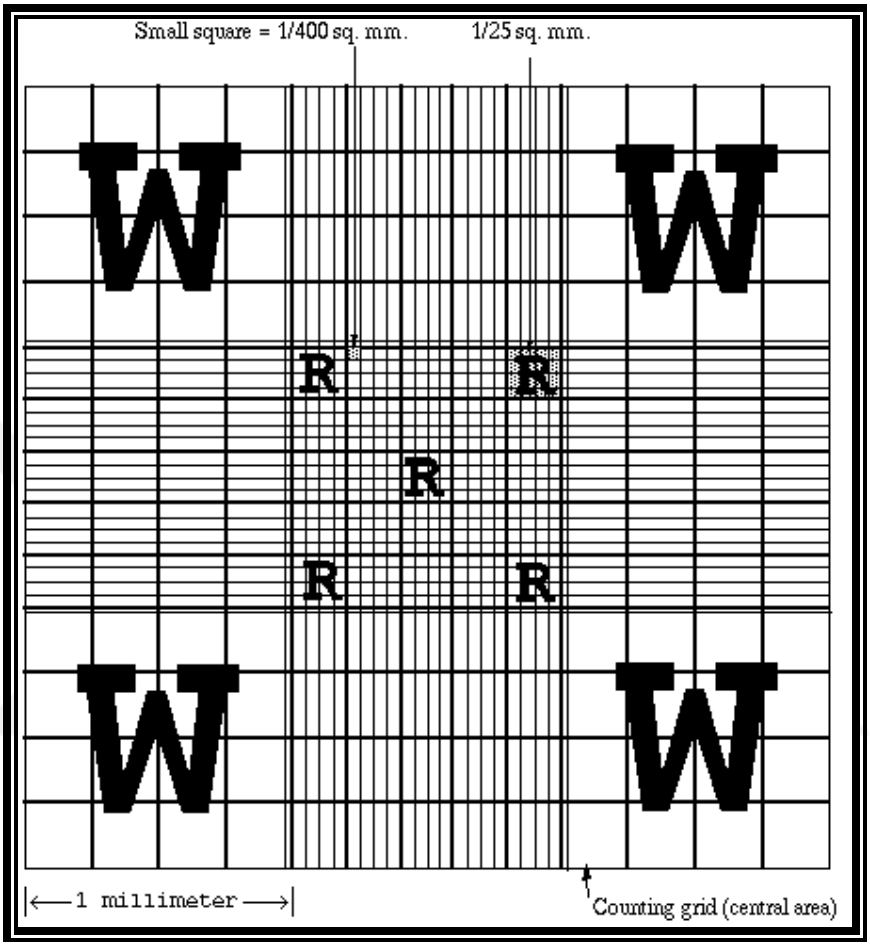
10. The WBCs count is reported as cell/mm3 from general relation which is

given as fallow ***(113)***.

WBCs (cell/mm3) = cells counted x 20 (dilution factor)/0.4mm (volume)

WBCs (cell/mm3) = cell count x 50 ***(115)***

The diluting fluid (2% acetic acid) solution is used to lyses the RBCs leaving only the WBCs visible [115].



Red cell count - Count the five (5) small squares indicated by "R".

White cell count - Count the four (4) large corner squares indicated by "W".

Figure (2.3): WBCs and RBCs counting slide [115]

2.4 OA outcome score

OA outcome score is developed as an instrument to assess the patients' opinion about their joint injury and associated problem, it is needed to be used for joint injury that can result in posttraumatic OA, also to be used over short and long time

intervals to assess changes from week to week induced by treatment (medication, operation, physical therapy) or over years, it can be used for patient with age (14-78) years and can be filled out by the patient in 10 minutes in the waiting room or filled out by the physician (researcher) through direct interview with the patient. The OOS sheet is shown in figure (2.4). Standardized answers options are given in 5 choice boxes, and each question gets a score from (0-4). A normalized score range from 100 which indicate no symptoms and 0 which indicates extreme symptoms is calculated as fallow

1. Symptoms score = 100-[Total score (S1-S7) x 100 /28]

2. Pain score = 100-[Total score (P1-P9) x 100 /36]

Knee injury and osteoarthritis outcome score (KOOS)

Pain

P 1 How often is your knee painful?

□ Never □ Monthly □ Weekly □ Daily □ Always

What degree of pain have you experienced the last week when...

P 2 Twisting / pivoting on your knee

□ None □ Mild □ Moderate □ Severe □ Extreme

P 3 Straightening knee fully

□ None □ Mild □ Moderate □ Severe □ Extreme

P 4 Bending knee fully

□ None □ Mild □ Moderate □ Severe □ Extreme

P 5 Walking on fl at surface

□ None □ Mild □ Moderate □ Severe □ Extreme

P 6 Going up or down stairs

□ None □ Mild □ Moderate □ Severe □ Extreme

P 7 At night while in bed

□ None □ Mild □ Moderate □ Severe □ Extreme

P 8 Sitting or lying

□ None □ Mild □ Moderate □ Severe □ Extreme

P 9 Standing upright

□ None □ Mild □ Moderate □ Severe □ Extreme

Symptoms

S 1 How severe is your knee stiffness after wakening in the morning?

□ None □ Mild □ Moderate □ Severe □ Extreme

S 2 How severe is your knee stiffness after sitting, lying, or resting later in the day?

□ None □ Mild □ Moderate □ Severe □ Extreme

S 3 Do you have swelling in your knee?

S 5 Does your knee catch or hangs up when moving?

□ Never □ Rarely □ Sometimes □ Often □ Always

S 6 Can you straighten your knee fully?

□ Always □ Often □ Sometimes □ Rarely □ Never

S 7 Can you bend your knee fully?

□ Always □ Often □ Sometimes □ Rarely □ Never

Figure (2.4) English version of KOOS survey sheet ***(121)***

2.5 Statistical analysis

1. The results were expressed as mean ± **SD** (slandered deviation).

2. Student T- test and **ANOVA** test were used to examine the degree of sig-

nificancy.

3. P value < 0.05 was considered significant.

3. RESULT

3.1 Elevation in serum malondialdehyde (MDA) level in osteoarthritic (OA)

patients as a function of age intervals (in years).

Table 3.1 and figure 3.1 showed that, Osteoarthritis produces significant elevation ($p< 0.05$) in serum MDA level as compared to the healthy control subjects as a function of age. The elevation level in serum MDA for age interval (30-39) year was (76.7%) and (90.5%) for (40-49) year, while in (50-59) year interval it was (66.1%) and (87.1%) for age interval (60-69) year. The maximum elevation level in serum MDA was (90.5%) at age interval of (40-49) year as compared to the healthy control subjects.

Table (3.1): Elevation in serum malondialdehyde (MDA) level in OA patients as compared to the healthy control subjects as a function of age.

Age intervals (years)	Number of subjects	Serum (MDA) level for healthy control subjects (μmol/L)	Number of patients	Serum (MDA) level for (OA) patients (μmol/L)
(30-39)	1	1.12	1	*(76.7%)* 1.98
(40-49)	4	1.06 ± 0.087	17	*(90.5%)* 2.02 ± 0.17 *
(50-59)	4	1.18 ± 0.058	25	*(66.1%)* 1.96 ± 0.1 *
(60-69)	2	1.09 ± 0.007	14	*(87.1%)* 2.04 ± 0.12 *
(70-79)	-	-	4	1.99 ± 0.081

Values are expressed as mean ± standard error of mean.

*** = Significant at (p< 0.05) as compared with the healthy control subjects.**

***( )* = values between two small brackets represent elevation level in serum**

MDA as compared with the healthy control subjects.

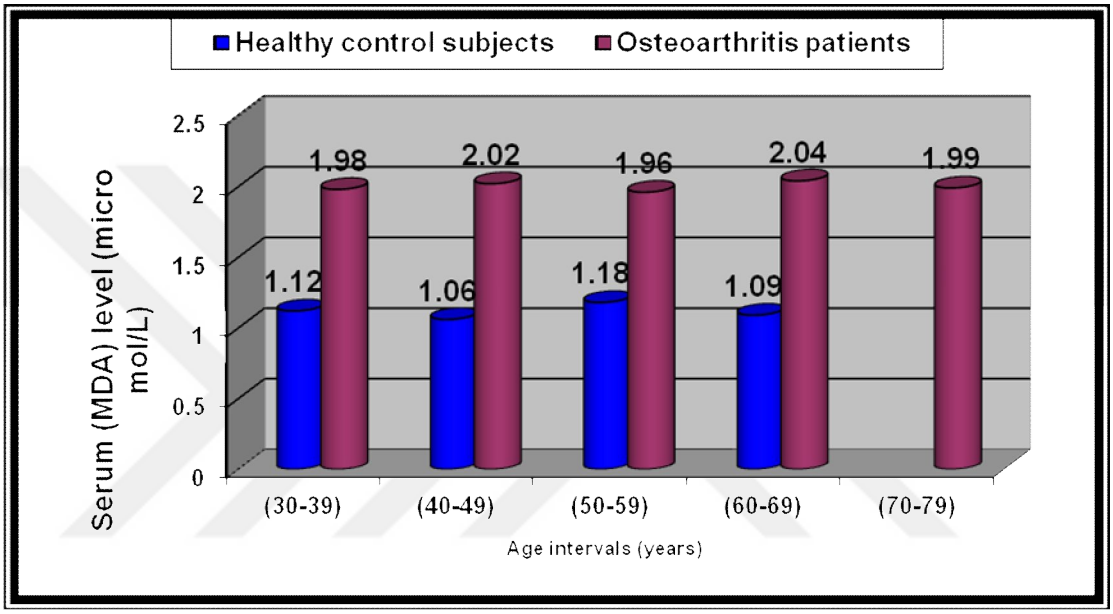


Figure (3.1): Elevation in serum malondialdehyde (MDA) level in OA patients as compared to the healthy control subjects as a function of age.

3.2 Elevation in serum malondialdehyde (MDA) level in OA patients as a function of body mass index (BMI).

Table 3.2 and figure 3.2 showed that, Osteoarthritis produces significant elevation ($p< 0.05$) in serum MDA level as compared to the healthy control subjects as a function of body mass index BMI. The elevation level in serum MDA for BMI (19.1-25.8) Kg/m^2 was (82.5%), and (76.5%) for BMI (25.8-27.3) Kg/m^2 as compared to the healthy control subjects. The maximum elevation level in serum MDA was (82.5%) at BMI (19.1-25.8) Kg/m^2 as compared to the healthy control subjects.

Table (3.2): Elevation in serum malondialdehyde (MDA) level in OA patients as compared to the healthy control subjects as a function of body mass index (BMI).

(BMI) intervals (Kg/m^2)	Number of subjects	Serum (MDA) level for healthy control subjects (μmol/L)	Number of patients	Serum (MDA) level for (OA) patients (μmol/L)
<19.1	-	-	1	2.2
(19.1-25.8)	8	1.09 ± 0.05	6	*(82.5%)* 1.99 ± 0.16 *
(25.8-27.3)	3	1.11 ± 0.045	9	*(76.5%)* 1.96 ± 0.11 *
(27.3-32.3)	-	-	22	2.0 ± 0.14
>32.3	-	-	23	1.77 ± 0.11

Values are expressed as mean ± standard error of mean.

*** = Significant at (p< 0.05) as compared with the healthy control subjects.**

***( )* = values between two small brackets represent elevation level in serum**

MDA as compared with the healthy control subjects.

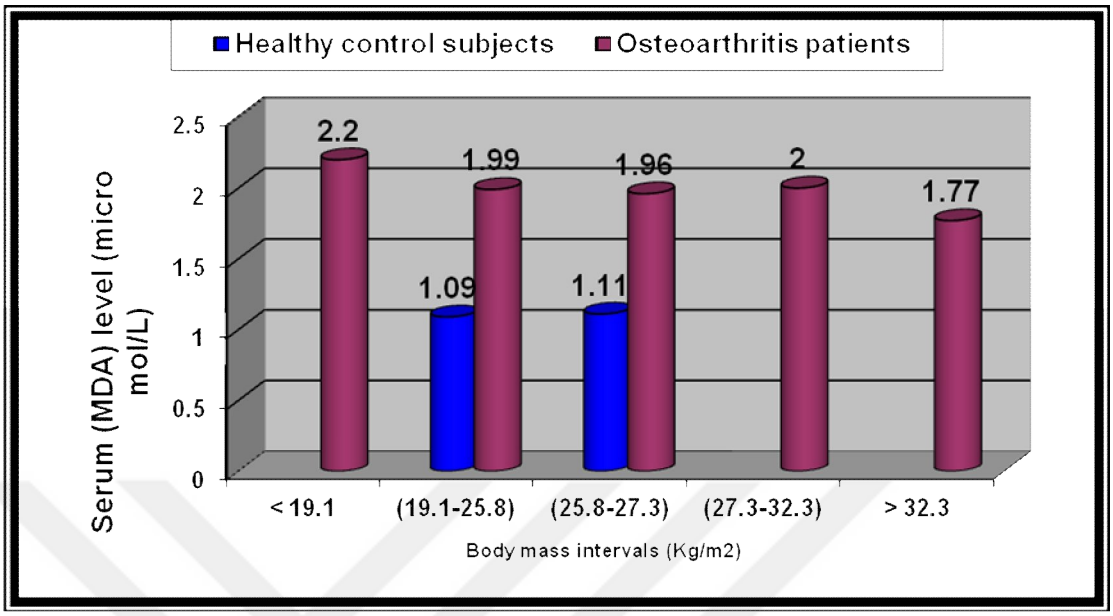


Figure (3.2): Elevation in serum malondialdehyde (MDA) level in OA patients as compared to the healthy control subjects as a function of body mass index (BMI).

3.3 Effect of vitamin E on serum malondialdehyde (MDA) level in OA patients treated with diclofenac sodium.

Table 3.3 and figure 3.3 showed significant elevation ($p< 0.05$) in serum MDA level in (group A and group B) as compared to the healthy control subjects (group E) at the baseline. The elevation level was (80% and 84.5%) respectively as compared with the healthy control subjects. Regarding OA patients treated with diclofenac sodium only (group A) the reduction level in serum MDA was (3%, 6%, 6.5%, and 6.5%) after 2, 4, 6, and 8 weeks of the treatment respectively as compared with baseline, while in OA patients treated with diclofenac sodium plus vitamin E (group B) the reduction level in serum MDA was (3.4%, 7.3%, 21.6%, and 35.4%) after 2, 4, 6, and 8 weeks of the treatment respectively as compared with baseline. The maximum reduction level in serum MDA in (group B) was 35.4% at the end of 8 weeks of the treatment compared to (6.5%) in (group A).

Table (3.3): Effect of vitamin E on serum malomdialdehyde (MDA) level in OA patients treated with diclofenac sodium.

Serum malondialdehyde (MDA) level (μmol/L)			
Treatment duration (wk)	**Group E N=11**	**Group A N=8**	**Group B N=20**
0 (baseline)	**1.1 ± 0.028**	**1.98 ± 0.11 $^{a\varphi}$**	**2.03 ± 0.12 $^{a\varphi}$**
2	**-**	***(3%)*** **1.92 ± 0.057 ^{ab}**	***(3.4%)*** **1.96 ± 0.1 ^{b}**
4	**-**	***(6%)*** **1.86 ± 0.081 ^{b}**	***(7.3%)*** **1.88 ± 0.062 ^{c}**
6	**-**	***(6.5%)*** **1.85 ± 0.102 ^{b}**	***(21.6%)*** **1.59 ± 0.109 ^{d*}**
8	**-**	***(6.5%)*** **1.85 ± 0.065 ^{b}**	***(35.4%)*** **1.31 ± 0.064 ^{e*}**

Values are expressed as mean ± standard error of mean.

Results with non-identical superscript (a, b, c, d, and e) within the same group were considered significantly different ($p< 0.05$).

*** = Significant ($p< 0.05$) as compared with group A (diclofenac sod. only).**

φ = Significant ($p< 0.05$) as compared with group E (healthy control subjects).

N = Number of patients.

Group E= Healthy control subjects.

<u>Group A</u>= Patients on diclofenac sodium only 100 mg/day.

<u>Group B</u>= Patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day.

***( )* = values between two small brackets represent reduction level in serum**

MDA as compared with the zero time (baseline).

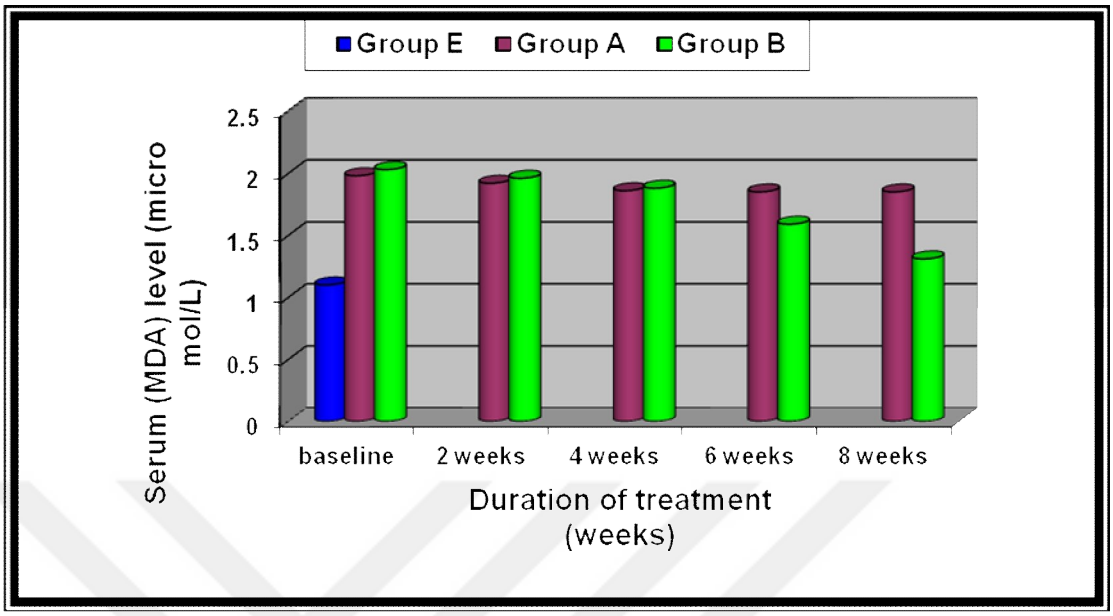


Figure (3.3): Effect of vitamin E on serum malondialdehyde (MDA) level in OA patients treated with diclofenac sodium.

Group E = Healthy control subjects.

Group A= Patients on diclofenac sodium only 100 mg/day.

Group B= Patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day.

3.4 Effect of vitamin C on serum malondialdehyde (MDA) in OA patients treated with diclofenac sodium.

Table 3.4 and figure 3.4 showed significant elevation ($p < 0.05$) in serum MDA level in (group A, and group C) at the baseline as compared to the healthy control subjects (group E).

The elevation level for (group A, and group C) at the baseline was (80% and 78.1%) respectively as compared with the healthy control subjects. Regarding OA patients treated with diclofnac sodium (group A) the reduction level in serum MDA was (3%, 6%, 6.5%, and 6.5%) after 2, 4, 6, and 8 weeks of the treatment respectively as compared with the baseline, while in OA patients treated with diclofenac sodium plus vitamin C (group C) the reduction level in serum MDA was (2.5%, 6.6%, 16.8%, and 24%) after 2, 4, 6, and 8 weeks of the treatment respectively as compared with the baseline.

The addition of vitamin C to OA regimen (diclofenac sodium) increase the reduction level in serum MDA to 24% at the end of 8 weeks of treatment and 6.5% when diclofenac sodium used alone as compared with the baseline, which slow the progression of cartilage destruction and improve signs and symptoms of OA patients.

Table (3.4): Effect of vitamin C on serum malondialdehyde (MDA) level in OA patients treated with diclofenac sodium.

Serum malondialdehyde (MDA) level (μmol/L)			
Treatment duration (wk)	**Group E N=11**	**Group A N=8**	**Group C N=19**
0 (baseline)	**1.1 ± 0.028**	**1.98 ± 0.11 $^{a\varphi}$**	**1.96 ± 0.16 $^{a\varphi}$**
2	**-**	***(3%)*** **1.92 ± 0.057 ^{ab}**	***(2.5%)*** **1.91 ± 0.13 ^{a}**
4	**-**	***(6%)*** **1.86 ± 0.081 ^{b}**	***(6.6%)*** **1.83 ± 0.11 ^{b}**
6	**-**	***(6.5%)*** **1.85 ± 0.102 ^{b}**	***(16.8%)*** **1.63 ± 0.094 ^{c*}**
8	**-**	***(6.5%)*** **1.85 ± 0.065 ^{b}**	***(24%)*** **1.48 ± 0.073 ^{d*}**

Values are expressed as mean ± standard error of mean.

Results with non-identical superscript (a, b, c, and d) within the same group were considered significantly different (p< 0.05).

*** = Significant (p< 0.05) as compared with group A (diclofenac sod. only).**

φ = Significant (p< 0.05) as compared with group E (healthy control subjects).

N = Number of patients.

Group E = Healthy control subjects.

Group A= Patients on diclofenac sodium only 100 mg/day.

Group C= Patients on diclofenac sodium 100mg/day plus vitamin C 1000 mg/day.

***( )* = values between two small brackets represent reduction level in serum**

MDA as compared with the zero time (baseline).

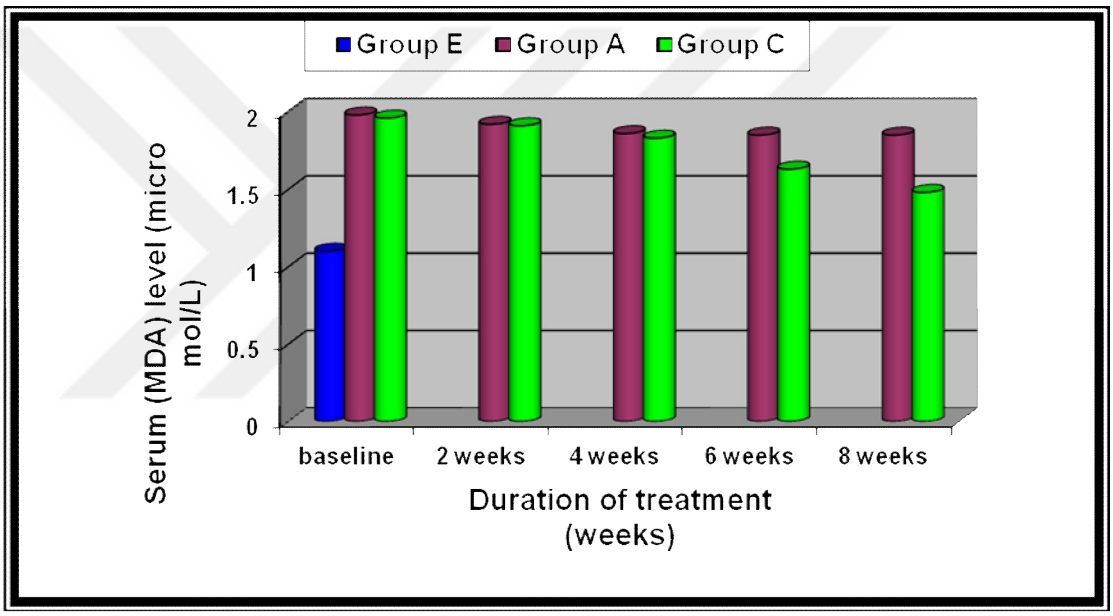


Figure (3.4): Effect of vitamin C on serum malondialdehyde (MDA) level in OA patients treated with diclofenac sodium.

Group E = healthy control subjects.

Group A= patients on diclofenac sodium only100 mg/day.

Group C= patients on diclofenac sodium 100 mg/day plus vitamin C 1000 mg/day.

3.5 Effect of vitamin E and vitamin C on serum malondialdehyde (MDA) level in OA patients treated with diclofenac sodium.

Table 3.5 and figure 3.5 showed significant elevation ($p< 0.05$) in serum MDA level in (group A and group D), the values were (80%, and 83.6%) respectively at the baseline as compared to the healthy control subjects (group E).

Regarding OA patients treated with diclofnac sodium only (group A) the reduction level in serum MDA was (3%, 6%, 6.5%, and 6.5%) after 2, 4, 6, and 8 weeks of treatment respectively as compared with baseline, while in OA patients treated with diclofenac sodium plus vitamin E and vitamin C (group D) the reduction level in serum MDA was (2.9%, 8.4%, 22.7%, and 37.6%) after 2 , 4, 6, and 8 weeks of the treatment respectively as compared with the baseline.

The use of combined antioxidants (vitamin E and C) in the treatment of OA patients will (may) increase the defense mechanism against ROS by slowing tissue destruction and improving patients outcome more than using single antioxidant, the reduction level in serum MDA in combined antioxidants was 37.6%, while in single antioxidant vitamin E or vitamin C the reduction level was (35.4%, and 24%) respectively at the end of 8 weeks of the treatment as compared with the baseline

Table (3.5): Effect of vitamin E and vitamin C on serum (MDA) level in OA patients treated diclofenac sodium.

Serum malondialdehyde (MDA) level (μmol/L)			
Treatment duration (wk)	**Group E N=11**	**Group A N=8**	**Group D N=14**
0 (baseline)	**1.1 ± 0.028**	**1.98 ± 0.11 $^{a\varphi}$**	**2.02 ± 0.072 $^{a\varphi}$**
2	**-**	***(3%)*** **1.92 ± 0.057 ^{ab}**	***(2.9%)*** **1.96 ± 0.076 ^{a}**
4	**-**	***(6%)*** **1.86 ± 0.081 ^{b}**	***(8.4%)*** **1.85 ± 0.032 ^{b}**
6	**-**	***(6.5%)*** **1.85 ± 0.102 ^{b}**	***(22.7%)*** **1.56 ± 0.14 ^{c*}**
8	**-**	***(6.5%)*** **1.85 ± 0.065 ^{b}**	***(37.6%)*** **1.26 ± 0.046 ^{d*}**

Values are expressed as mean ± standard error of mean.

Results with non-identical superscript (a, b, c and d) within the same group were considered significantly different (p< 0.05).

*** = Significant (p< 0.05) as compared with group A (diclofenac sod. only).**

φ = Significant (p< 0.05) as compared with group E (healthy control subjects).

N = Number of patients.

<u>Group E</u> = Healthy control subjects.

Group A= Patients on diclofenac sodium only 100 mg/day.

Group D= Patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day and vitamin C 1000 mg/day.

***( )* = values between two small brackets represent reduction level in serum MDA as compared with the zero time (baseline).**

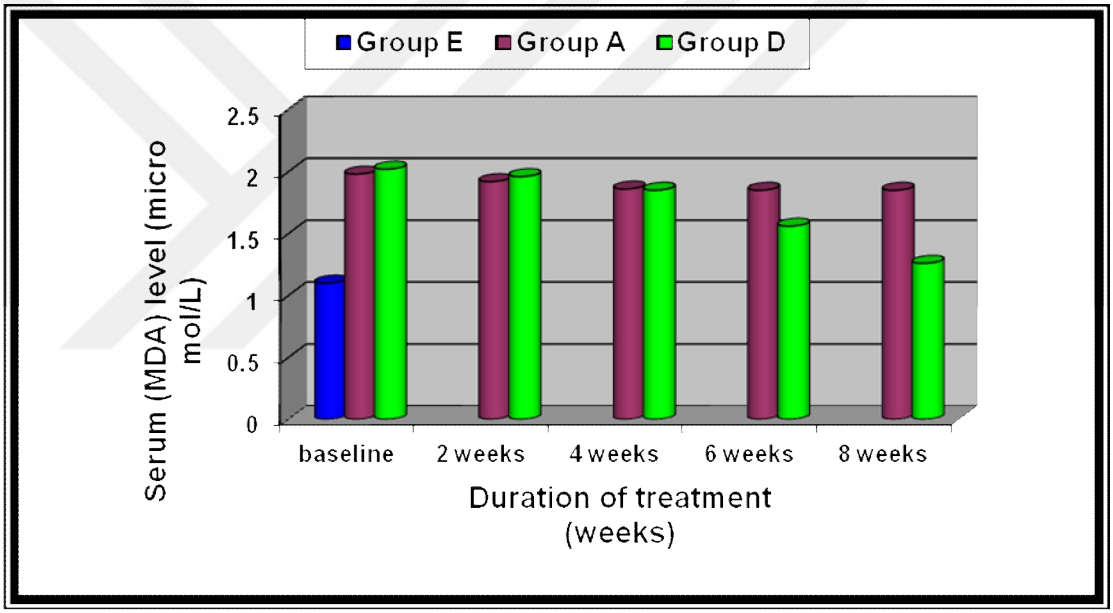


Figure (3.5): Effect of vitamin E and vitamin C on serum malondialdehyde (MDA) level in OA patients treated with diclofenac sodium.

Group E = Healthy control subjects.

Group A= Patients on diclofenac sodium only 100 mg/day.

Group D= Patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day and vitamin C 1000 mg/day.

3.6 The effect of different antioxidants on serum MDA level in OA patients

treated with diclofenac sodium after 8 weeks of the treatment

Figure 3.6 showed the reduction in MDA level using different antioxidants (vitamin E and/or vitamin C) in OA patients treated with diclofenac sodium, adding of vitamin E to patients therapy the MDA level reduction was 1.31 and in vitamin C was 1.84, while using combination of vitamin E and C the values were reduced to 1.26 after 8 week of treatment.

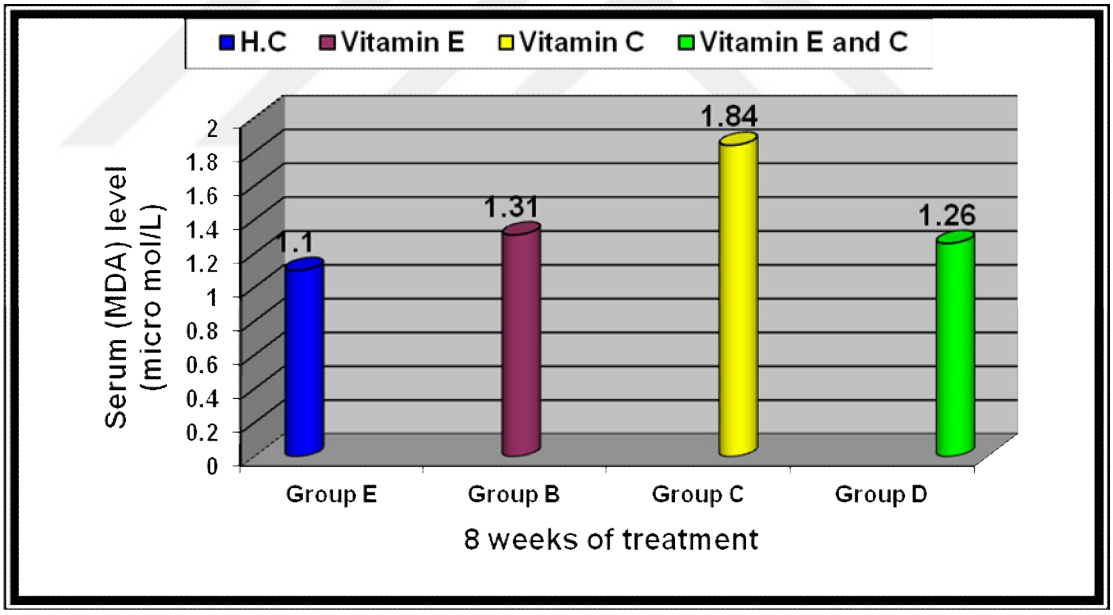


Figure (3.6): The effect of different antioxidants on serum MDA level in OA patients treated with diclofenac sodium after 8 weeks of treatment

3.7 Reduction in serum glutathione (GSH) level in OA patients as a function of age intervals (in years).

Table 3.6 and figure 3.7 showed that, Osteoarthritis produces significant reduction ($p < 0.05$) in serum GSH level as compared to the healthy control subjects as a function of age. The reduction level in serum GSH is for age intervals (30-39) year was 55.2% and 68.6% for (40-49) year, while in (50-59) year intervals was 63.6% and 66% for age intervals (60-69) year.

Table 3.6 and figure 3.7 showed the maximum reduction level in serum GSH was 68.6% at age intervals (40-49) years as compared to the healthy control subjects.

Table (3.6): Reduction in serum glutathione (GSH) level in OA patients as a function of age intervals (in years)

Age intervals (years)	Number of subjects	Serum (GSH) level for healthy control subjects (μmol/L)	Number of patients	Serum (GSH) level for (OA) patients (μmol/L)
(30-39)	1	1.05	1	*(55.2%)* 0.47
(40-49)	4	0.99 ± 0.043	17	*(68.6%)* 0.31 ± 0.076 *
(50-59)	4	0.99 ± 0.057	25	*(63.6%)* 0.36 ± 0.11 *

(60-69)	2	1.03 ± 0.25	14	*(66%)* 0.35 ± 0.095 *
(70-79)	-	-	4	0.27 ± 0.092

Values are expressed as mean ± standard error of mean.

*** = Significant at (p< 0.05) as compared with healthy control subjects.**

***( )* = values between two small brackets represent reduction level in serum**

GSH as compared with the healthy control subjects.

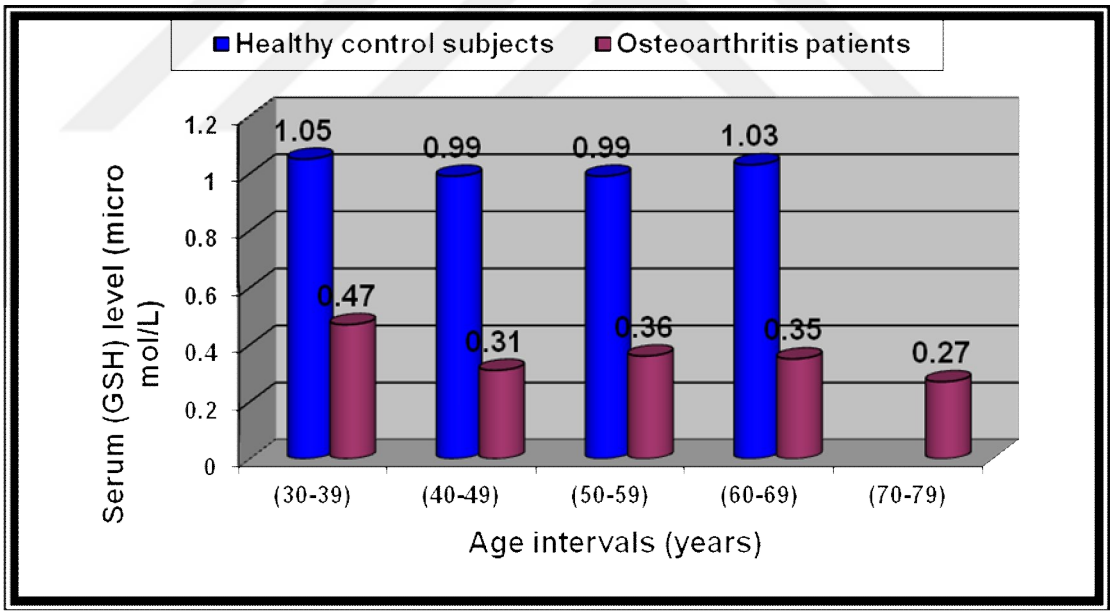


Figure (3.7): Reduction in serum glutathione (GSH) level in OA patients as a function of age intervals (in years).

3.8 Reductions in serum glutathione (GSH) level in OA patients as a function of body mass index (BMI).

Table 3.7 and figure 3.8 showed that, Osteoarthritis produces significant reduction ($p< 0.05$) in serum GSH level as compared to the healthy control subjects as a function of body mass index (BMI). The reduction level in serum GSH is for BMI (19.1-25.8) Kg/m^2 was 66%, while for BMI (25.8-27.3) Kg/m^2 the reduction level was 64.1%.

Table 3.7 and figure 3.8 show that, the maximum reduction level in serum GSH was at body mass index (19.1-25.8) Kg/m^2 which is 66% as compared to the healthy control subjects.

Table (3.7): Reductions in serum glutathione (GSH) level in OA patients as a function of body mass index (BMI)

(BMI) intervals (Kg/m^2)	Number of subjects	Serum (GSH) level for healthy control subjects (µmol/L)	Number of patients	Serum (GSH) level for (OA) patients (µmol/L)
<19.1	-	-	1	0.41
(19.1-25.8)	8	1.03 ± 0.084	6	*(66%)* 0.35 ± 0.11 *
(25.8-27.3)	3	0.92 ± 0.034	9	*(64.1%)*0.33 ± 0.095 *
(27.3-32.3)	-	-	22	0.36 ± 0.11

>32.3	**-**	**-**	**23**	**0.31 ± 0.087**

Values are expressed as mean ± standard error of mean.

*** = Significant (p< 0.05) as compared with (healthy control subjects).**

***( )* = values between two small brackets represent reduction level in serum**

GSH as compared with the healthy control subjects.

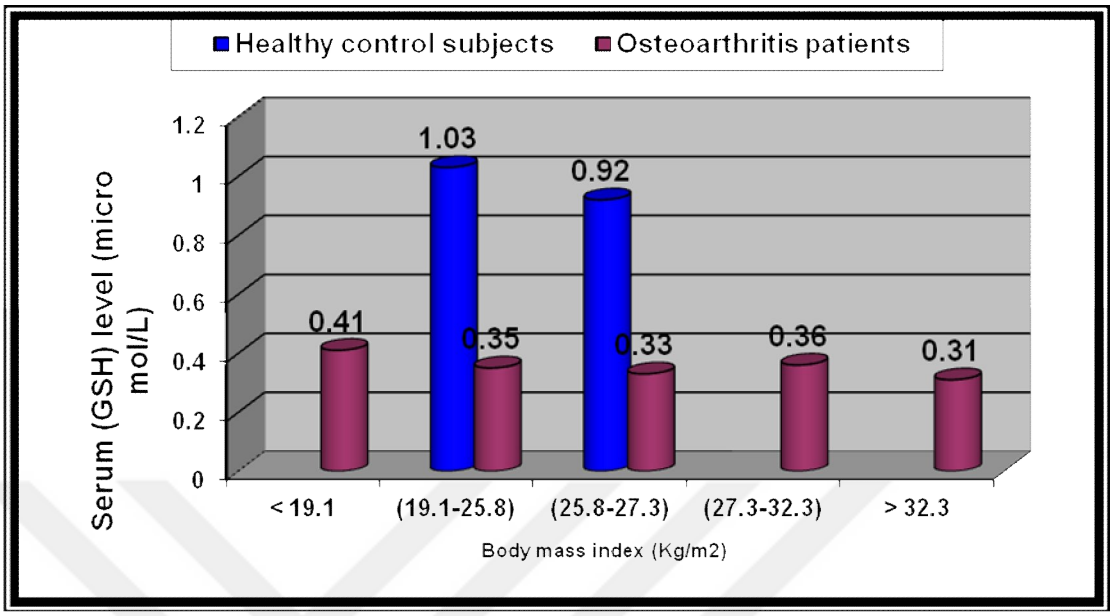


Figure (3.8): Reduction in serum glutathione (GSH) level in OA patients as a function of body mass index (BMI).

3.9 Effect of vitamin E on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Table 3.8 and figure 3.9 showed significant reduction ($p < 0.05$) in serum GSH level in (group A and group B) at the baseline as compared to the healthy control subjects, the reduction were (68%, and 64%) respectively. Regarding OA patients treated with diclofenac sodium only (group A) the elevation level in serum GSH was (3.1%, 9.3%, 12.5%, and 15.6%) after 2 , 4, 6, and 8 weeks of the treatment respectively as compared with the baseline, while in (group B) the elevation level in serum GSH was (5.5%, 11.1%, 97.2%, and 152%) after 2, 4, 6, and 8 weeks of the treatment respectively as compared with baseline. **The maximum elevation** level in serum GSH for group A and group B were (15.6% and 152%) respectively after 8 weeks of the treatment as compared to the baseline.

Table (3.8): Effect of vitamin E on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Serum glutathione (GSH) level μmol/L			
Treatment duration (wk)	**Group E N=11**	**Group A N=8**	**Group B N=20**
0 (baseline)	**1.0 ± 0.089**	**0.32 ± 0.1 [aφ]**	**0.36 ± 0.13 [aφ]**
2	**-**	***(3.1%)*** **0.33 ± 0.046 [a]**	***(5.5%)*** **0.38 ± 0.12 [a]**
4	**-**	***(9.3%)*** **0.35 ± 0.075 [a]**	***(11.1%)*** **0.4 ± 0.13 [a]**
6	**-**	***(12.5%)*** **0.36 ± 0.084 [a]**	***(97.2%)*** **0.71 ± 0.1 [b*]**
8	**-**	***(15.6%)*** **0.37 ± 0.068 [a]**	***(152%)*** **0.91 ± 0.097 [c*]**

Values are expressed as mean ± standard error of mean.

Results with non-identical superscript (a, b, and c) within the same group were considered significantly different (p< 0.05).

*** = Significant (p< 0.05) as compared with group A (diclofenac sod. only).**

φ = Significant (p< 0.05) as compared with group E (healthy control subjects).

N = Number of patients.

Group E = Healthy control subjects.

<u>Group A</u>= Patients on diclofenac sodium only 100 mg/day.

<u>Group B</u>= Patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day.

***( )* = values between two small brackets represent elevation level in serum**

GSH as compared with the zero time (baseline).

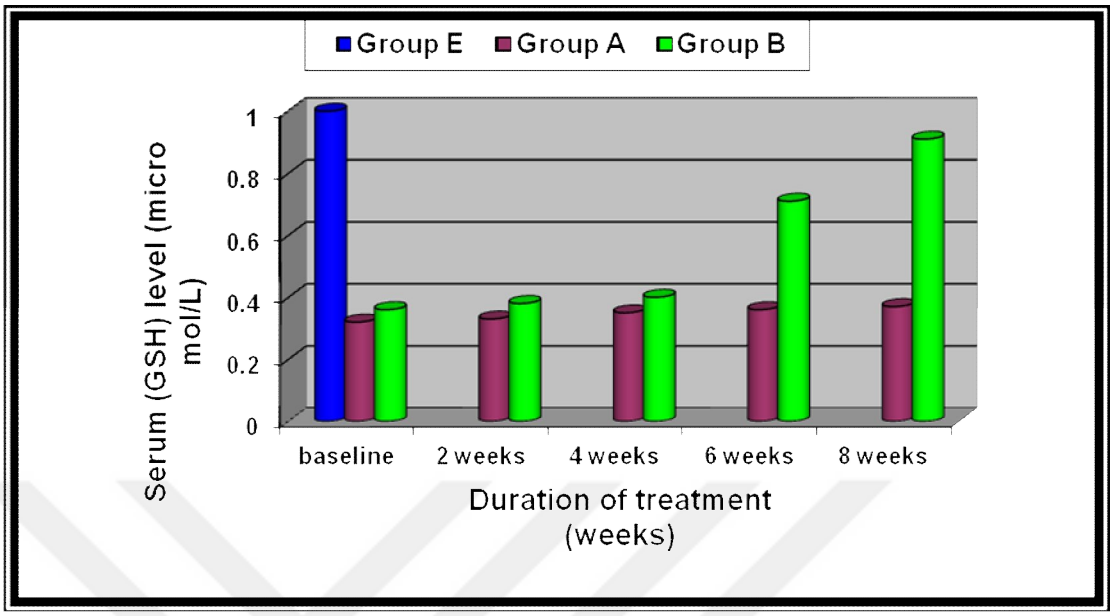


Figure (3.9): Effect of vitamin E on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Group E = healthy control subjects.

Group A= patients on diclofenac sodium only100 mg/day.

Group B= patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day.

3.10 Effect of vitamin C on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Table 3.9 and figure 3.10 showed significant reduction ($p < 0.05$) in serum GSH level in (group A and group C) at the baseline as compared with the healthy control subjects the reduction level was (68%, and 66%) respectively. Regarding OA patients treated with diclofenac sodium only (group A) the elevation level in serum GSH was (3.1%, 9.3%, 12.5%, and 15.6%) after 2, 4, 6, and 8 weeks of treatment respectively as compared with the baseline, while in (group C), the elevation level was (2.9%, 11.7%, 79.4%, and 123%) after 2, 4, 6, and 8 weeks of treatment respectively as compared with the baseline.

The addition of vitamin C to the OA treatment regimen (diclofenac sodium) give maximum elevation in defense mechanism against ROS through elevation in serum GSH level of 123% compared to 15.6% when diclofenac sodium used alone at the end of 8 weeks of treatment as compared with baseline.

Table (3.9): Effect of vitamin C on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Serum glutathione (GSH) level μmol/L			
Treatment duration (wk)	**Group E N=11**	**Group A N=8**	**Group C N=19**
0 (baseline)	**1.0 ± 0.089**	**0.32 ± 0.1 $^{a\varphi}$**	**0.34 ± 0.068 $^{a\varphi}$**
2	**-**	***(3.1%)*** **0.33 ± 0.046 ^{a}**	***(2.9%)*** **0.35 ± 0.065 ^{a}**
4	**-**	***(9.3%)*** **0.35 ± 0.075 ^{a}**	***(11.7%)*** **0.38 ± 0.081 ^{a}**
6	**-**	***(12.5%)*** **0.36 ± 0.084 ^{a}**	***(79.4%)*** **0.61 ± 0.098 ^{b*}**
8	**-**	***(15.6%)*** **0.37 ± 0.068 ^{a}**	***(123%)*** **0.76 ± 0.1 ^{c*}**

Values are expressed as mean ± standard error of mean.

Results with non-identical superscript (a, b, and c) within

the same group were considered significantly different ($p < 0.05$).

*** = Significant ($p < 0.05$) as compared with group A (diclofenac sod. only).**

φ = Significant ($p < 0.05$) as compared with group E (healthy control subjects).

N = Number of patients.

Group E = Healthy control subjects.

Group A= Patients on diclofenac sodium only 100 mg/day.

Group C= Patients on diclofenac sodium 100 mg/day plus vitamin C 1000 mg/day.

***( )* = values between two small brackets represent elevation level in serum**

GSH as compared with the zero time (baseline).

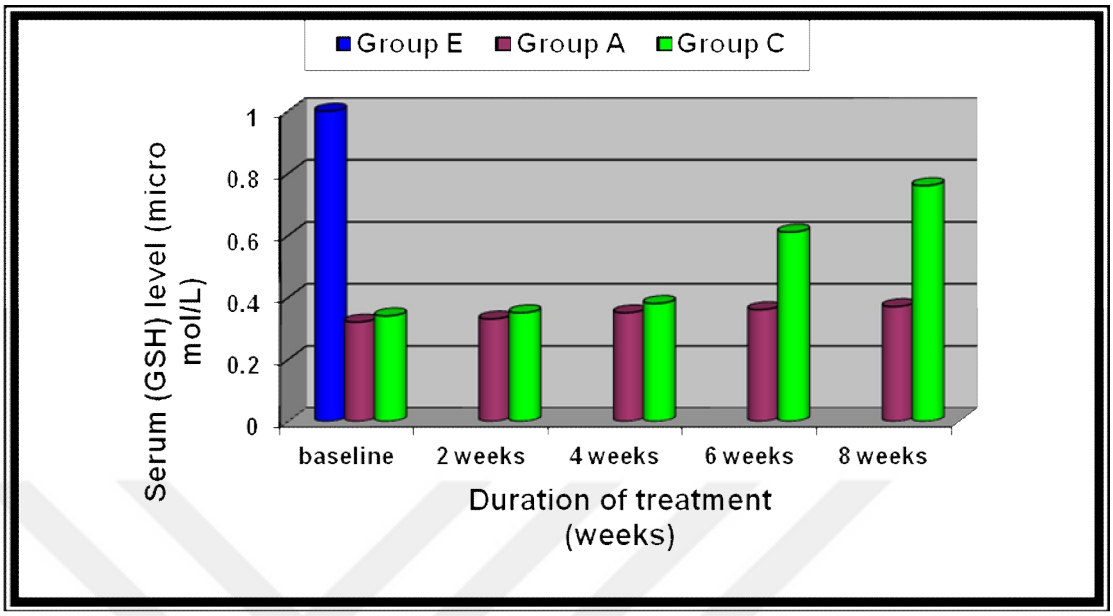


Figure (3.10) Effect of vitamin C on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Group E = Healthy control subjects.

Group A= Patients on diclofenac sodium only 100 mg/day.

Group C= Patients on diclofenac sodium 100 mg/day plus vitamin C 100 mg/day.

3.11 Effect of vitamin E and vitamin C on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Table 3.10 and figure 3.11 showed significant reduction ($p < 0.05$) in serum GSH level at the baseline in (group A and group D) as compared to the healthy control subjects, the reduction level was (68%, and 70%) respectively. Regarding OA patients treated with diclofenac sodium only (group A) the elevation level in serum GSH was (3.1%, 9.3%, 12.5%, and 15.6%) after 2 , 4, 6, and 8 weeks of the treatment respectively as compared with the baseline, while (group D) the elevation level in serum GSH was (6.6%, 16.6%, 166%, and 223%) after 2, 4, 6, and 8 weeks of the treatment respectively as compared with the baseline. The using of combined antioxidants (vitamin E and C) **effect** the level of serum GSH (223%), compared with (152%, and 123%) when vitamin E or vitamin C used alone respectively.

Table (3.10): Effect of vitamin E and vitamin C on serum glutathione (GSH) level in OA patients treated with diclofenac sodium.

Serum glutathione (GSH) level μmol/L			
Treatment duration (wk)	**Group E N=11**	**Group A N=8**	**Group D N=14**
0 (baseline)	**1.0 ± 0.089**	**0.32 ± 0.1 $^{a\varphi}$**	**0.30 ± 0.079 $^{a\varphi}$**
2	**-**	***(3.1%)*** **0.33 ± 0.046 ^{a}**	***(6.6%)*** **0.32 ± 0.07 ^{a}**
4	**-**	***(9.3%)*** **0.35 ± 0.075 ^{a}**	***(16.6%)*** **0.35 ± 0.063 ^{a}**
6	**-**	**(12.5%) 0.36 ± 0.084 ^{a}**	***(166%)*** **0.80 ± 0.06 ^{b*}**
8	**-**	***(15.6%)*** **0.37 ± 0.068 ^{a}**	***(223.3%)*** **0.97 ± 0.097 ^{c*}**

Values are expressed as mean ± standard error of mean. Results with non-identical superscript (a, b, and c) within the same group were considered significantly different (p<

0.05).

*** = Significant (p< 0.05) as compared with group A (diclofenac sod. only).**

φ = Significant (p< 0.05) as compared with group E (healthy control subjects).

N = Number of patients.

Group E = Healthy control subjects.

Group A= Patients on diclofenac sodium only 100 mg/day.

Group D= Patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day and

vitamin C 1000 mg/day.

***( )* = values between two small brackets represent elevation level in serum**

GSH as compared with the zero time (baseline).

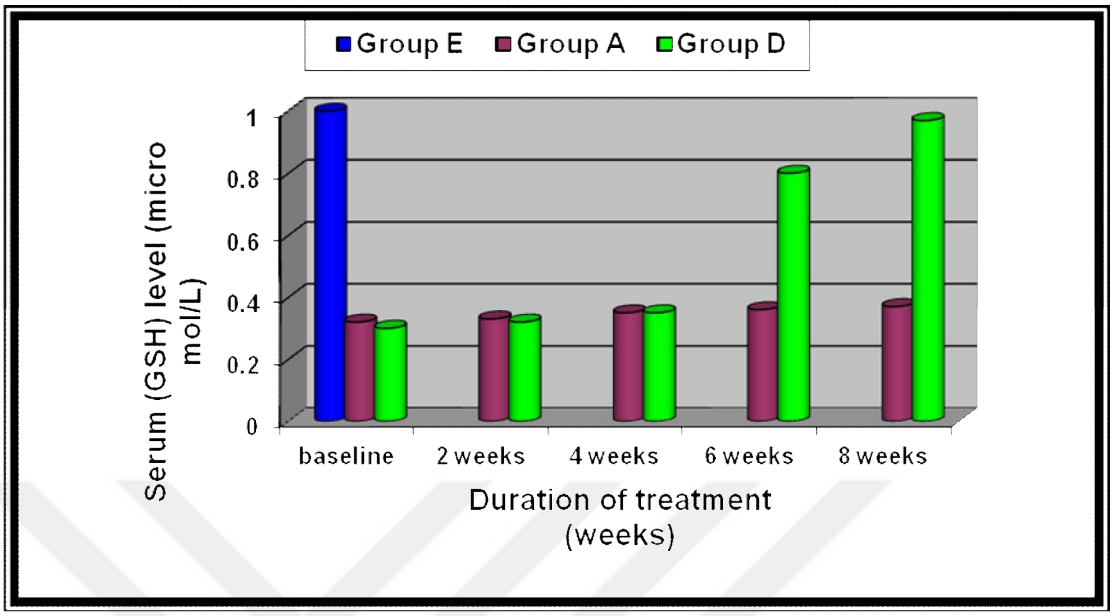


Figure (3.11) Effect of vitamin E and vitamin C on serum glutathione (GSH) level in OA patients treated with diclofenac sodium).

<u>Group E</u> = Healthy control subjects.

<u>Group A</u>= Patients on diclofenac sodium only 100 mg/day.

<u>Group D</u>= Patients on diclofenac sodium 100 mg/day plus vitamin E 800 mg/day and vitamin C 1000 mg/day.

3.12 The effect of different antioxidants on serum GSH level in OA patients treated with diclofenac sodium after 8 weeks of the treatment.

Figure 3.12 showed that, the GSH level increased by adding antioxidants (vitamin E and/or vitamin C) to diclofenac therapy in which the GSH level was increased to 0.97 μmol/l in case of using combined antioxidants compared with (0.91, and 0.76) μmol/L when vitamin E or vitamin C added respectively after 8 weeks of the treatment.

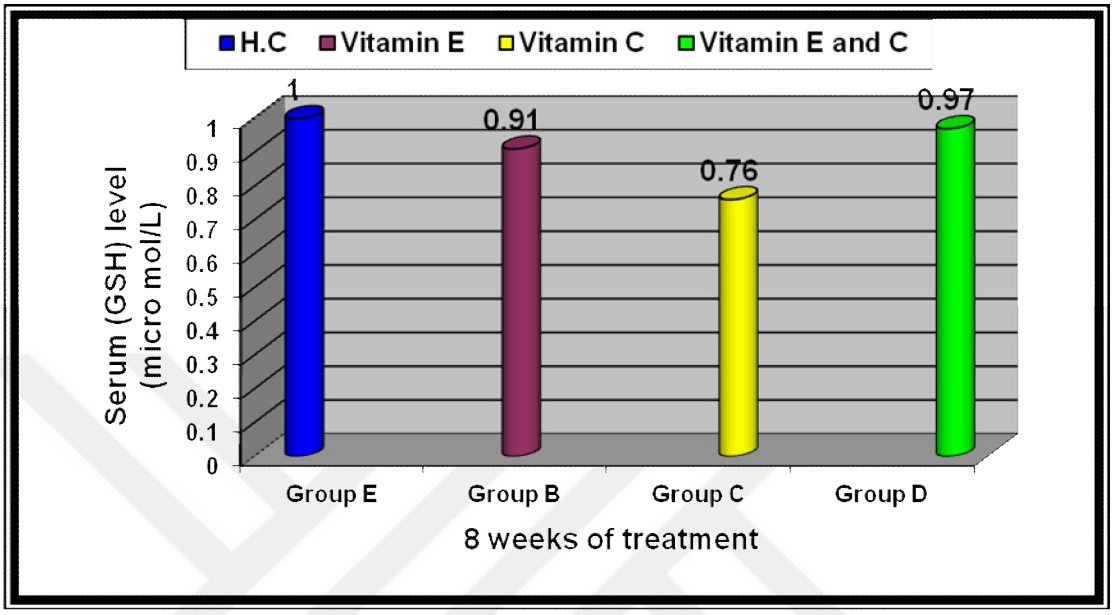


Figure (3.12): comparison the treatment of osteoarthritis patients with Antioxidants on serum GSH level at the end of 8 weeks

3.13 Effect of C-reactive protein (CRP) in OA patients on other laboratory

tests.

Table 3.11 showed, OA patients with **negative CRP** produced significant **elevation** (39%, 80.9%, and 10.1%) in ESR, MDA, and WBCs, respectively, in addition to significant reduction (8.7%, and 67%) in Hgb, and GSH respectively, while OA patients with positive CRP showed significant elevation (128%, 85.4%, and 13.7%) in ESR, MDA, and WBCs respectively, in addition to significant reduction (13.4%, and 63%) in Hgb, and GSH respectively as compared with the healthy control subjects.

Table (3.11): Effect of CRP test in OA patients on other laboratory tests.

Laboratory tests	Group E N=11	-Ve CRP test N=54	+Ve CRP test N=7
ESR (mm/hr)	19.4 ± 4.51	26.98 ± 9.6 *	44.28 ± 9.42 *φ
RF (IU/mL)	100% (-ve)	96.3% (-ve)	100% (-ve)
Hgb (gm/dL)	14.9 ± 1.19	13.6 ± 0.94 *	12.9 ± 0.75 *φ
MDA (µmol/L)	1.1 ± 0.069	1.99 ± 0.14 *	2.04 ± 0.106 *
GSH (µmol/L)	1.0 ± 0.089	0.33 ± 0.10 *	0.37 ± 0.05 *
WBCs (No. x 10^9/L)	5.8 ± 0.57	6.39 ± 0.102 *	6.6 ± 1.19 *

Values are expressed as mean ± standard error of mean.

*** = Significant (p< 0.05) as compared with group E (healthy**

control subjects).

φ = Significant (p< 0.05) as compared with (–Ve CRP) group.

N = Number of patients.

Group E = Healthy control subjects.

3.14 Effect of rheumatoid factor (RF) in OA patients on other laboratory tests.

Table 3.12 showed that, OA patients with **negative RF produce significant elevation** (53.8%, 80.9%, and 9.8%) in ESR, MDA, and WBCs respectively, in addition to significant reduction (9.3%, and 66%) in Hgb, and GSH respectively, while in OA patients with positive RF produces significant elevation (13.4%, 90%, and 33.6%) in ESR, MDA, and WBCs respectively in addition to significant reduction 63% in GSH level.

Table (3.12): Effect of RF in OA patients on other laboratory tests

Laboratory tests	Group E N=11	-Ve RF test N=59	+Ve RF test N=2
ESR (mm/hr)	19.4 ± 4.51	29.84 ± 9.5 *	22.0 ± 14.14 *
CRP (mg/L)	100% (-ve)	88.14% (-ve)	100% (-ve)
Hgb (gm/dL)	14.9 ± 1.19	13.5 ± 0.91 *	14.9 ± 1.25
MDA(µmol/L)	1.1 ± 0.069	1.99 ± 0.14 *	2.09 ± 0.028 *

GSH (µmol/L)	**1.0 ± 0.089**	**0.34 ± 0.10 ***	**0.37 ± 0.05 ***
WBCs (No. x 10^9/L)	**5.8 ± 0.57**	**6.37 ± 0.103 ***	**7.75 ± 1.34 ***

Values are expressed as mean ± standard error of mean.

*** = Significant (p< 0.05) as compared with group E (healthy control subjects).**

φ = Significant (p< 0.05) as compared with (–Ve RF) group.

N = Nmber of patients.

<u>Group E</u> = Healthy control subjects.

3.15 Effect of different antioxidants on symptoms and pain score in OA patients.

All OA patients showed low symptoms and pain score at the baseline.

Table 3.13 and 3.14 showed that, treatment of OA patients with diclofenac sodium result in significant elevation (p< 0.05) in **symptoms score** (7.1%, 8.7%, 13.4%, and 19.1%) after 2, 4, 6, and 8 week of the treatment respectively as compared with the zero time (baseline). The maximum elevation in symptoms score 19.1% after 8 weeks of the treatment. Significant elevation (p< 0.05) in the pain score (10%, 16%, 24.1%, and 30%) after 2, 4, 6, and 8 week of the treatment respectively as compared with the zero time (baseline). The maximum elevation in pain score was 30% after 8 weeks of the treatment.

Treatment of OA patients with diclofenac sodium plus

vitamin E result in significant elevation in the symptoms score (6.4%, 14.1%, 24.5%, and 35.2%) after 2, 4, 6, and 8 weeks of the treatment respectively as compared with the zero time (baseline). The maximum elevation in symptoms score was 35.2% after 8 weeks of the treatment. Significant elevation in pain score (11.6%, 19.7%, 26.9, and 37.9%) after 2, 4, 6, and 8 weeks of the treatment respectively, the maximum elevation in pain score was 37.9% after 8 weeks of the treatment.

Table 3.15, and 3.16 showed that, treatment of OA patients with diclofenac sodium plus vitamin C result in significant elevation in symptoms score (4.8%, 13.8%, 22.2%, and 32%) after 2, 4, 6, and 8 week of the treatment respectively, the maximum elevation in symptoms score was 32% after 8 weeks of the treatment. Significant elevation in pain score (4.9%, 16%, 26.1%, and 35%) after 2, 4, 6, and 8 week of the treatment respectively, the maximum elevation in pain score was 35% after 8 weeks of the treatment.

Table 3.17, and 3.18 showed that, treatment of OA patients with diclofenac sodium plus vitamin E and C resulting in significant elevation in symptoms score (11.1%, 22%, 30.6%, and 41.2%) after 2, 4, 6, and 8 weeks of the treatment respectively, the maximum elevation in symptoms score was 41.2% after 8 weeks of the treatment. Significant elevation in pain score (12.1%, 24.8%, 36.2%, and 45%) after 2, 4, 6, and 8 weeks of the treatment respectively, the maximum elevation in pain score was 45% after 8 weeks of the teatment.

Figure 3.13 and Figure 3.14 showed improvement in pain and symptoms score, the elevation level in pain score was (71.1, and 68.2) for group B and group C respectively, and (71.7, and 68.4) respectively for symptoms score. The symptoms and pain score were increased to (78.8, and 76.6) when combined antioxidants used respectively after 8 weeks of the treatment.

Table (3.13): Effect of vitamin E on symptoms score in OA patients treated with diclofenac sodium

Symptoms score		
Treatment duration (wk)	**Group A N=8**	**Group B N=20**
0 (baseline)	**55.8 ± 1.99**	**53.0 ± 2.58**
2	***(7.1%)*** **59.8 ± 2.12** *	***(6.4%)*** **56.4 ± 2.19** *
4	***(8.7%)*** **60.7 ± 2.13** *	***(14.1%)*** **60.5 ± 2.25** *
6	***(13.4%)*** **63.3 ± 1.98** *	***(24.5%)*** **66.0 ± 2.0** *

8	***(19.1%)*** **66.5 ± 1.84** *	***(35.2%)*** **71.7 ± 1.44** *

Table (3.14): Effect of vitamin E on pain score in OA patients treated with diclofenac sodium.

pain score		
Treatment duration (wk)	**Group A N=8**	**Group B N=20**
0 (baseline)	**51.7 ± 3.2**	**51.6 ± 2.08**
2	***(10%)*** **56.9 ± 3.07** *	***(11.6%)*** **57.7 ± 1.75** *
4	***(16%)*** **60.4 ± 3.05** *	***(19.7%)*** **61.8 ± 1.71** *
6	***(24.1%)*** **64.2 ± 2.85** *	***(26.9%)*** **65.5 ± 1.5** *
8	***(30%)*** **67.3 ± 2.91** *	***(37.9%)*** **71.1 ± 1.49** *

Values are expressed as mean ± standard error of mean.

All values are significant different as compared with the zero time (baseline).

( ) **= values between two small brackets represent elevation level in symptoms**

or pain score as compared with the zero time (baseline).

Table (3.15): Effect of vitamin C on symptoms score in OA patients treated with diclofenac sodium

Symptoms score

Treatment duration (wk)	Group A N=8	Group C N=19
0 (baseline)	55.8 ± 1.99	51.8 ± 2.57
2	*(7.1%)* 59.8 ± 2.12 *	*(4.8%)* 54.3 ± 2.14 *
4	*(8.7%)* 60.7 ± 2.13 *	*(13.8%)* 59.0 ± 2.29 *
6	*(13.4%)* 63.3 ± 1.98 *	*(22.2%)* 63.3 ± 2.32 *
8	*(19.1%)* 66.5 ± 1.84 *	*(32%)* 68.4 ± 2.06 *

Table (3.16): Effect of vitamin C on pain score in OA patients treated with diclofenac sodium.

pain score		
Treatment duration (wk)	Group A N=8	Group C N=19
0 (baseline)	51.7 ± 3.2	50.5 ± 2.79
2	*(10%)* 56.9 ± 3.07 *	*(4.9%)* 53.0 ± 2.41 *
4	*(16%)* 60.4 ± 3.05 *	*(16%)* 58.6 ± 2.24 *
6	*(24.1%)* 64.2 ± 2.85 *	*(26.1%)* 63.7 ± 2.17 *
8	*(30%)* 67.3 ± 2.91 *	*(35%)* 68.2 ± 2.19 *

Values are expressed as mean ± standard error of mean.

All values are significant different as compared with the zero

time (baseline).

***( )* = values between two small brackets represent elevation level in symptoms**

or pain score as compared with the zero time (baseline).

Table (3.17): Effect of vitamin E and C on symptoms score in OA patients treated with diclofenac sodium

Symptoms score		
Treatment duration (wk)	**Group A N=8**	**Group D N=14**
0 (baseline)	**55.8 ± 1.99**	**55.8 ± 3.12**
2	***(7.1%)*** **59.8 ± 2.12** *	***(11.1%)*** **62.0 ± 2.81** *
4	***(8.7%)*** **60.7 ± 2.13** *	***(22%)*** **68.1 ± 2.43** *
6	***(13.4%)*** **63.3 ± 1.98** *	***(30.6%)*** **72.9 ± 2.37** *
8	***(19.1%)*** **66.5 ± 1.84** *	***(41.2%)*** **78.8 ± 2.07** *

Table

(3.18): Effect of vitamin E and C on symptoms score in OA patients treated with diclofenac sodium

pain score		
Treatment duration (wk)	**Group A N=8**	**Group D N=14**

0 (baseline)	**51.7 ± 3.2**	**52.7 ± 3.08**
2	***(10%)*** **56.9 ± 3.07** *	***(12.1%)*** **59.1 ± 2.7** *
4	***(16%)*** **60.4 ± 3.05** *	***(24.8%)*** **65.8 ± 1.97** *
6	***(24.1%)*** **64.2 ± 2.85** *	***(36.2%)*** **71.8 ± 1.83** *
8	***(30%)*** **67.3 ± 2.91** *	***(45%)*** **76.6 ± 1.6** *

Values are expressed as mean ± standard error of mean.

All values are significant different as compared with the zero time (baseline).

( ) **= values between two small brackets represent elevation level in symptoms**

or pain score as compared with the zero time (baseline).

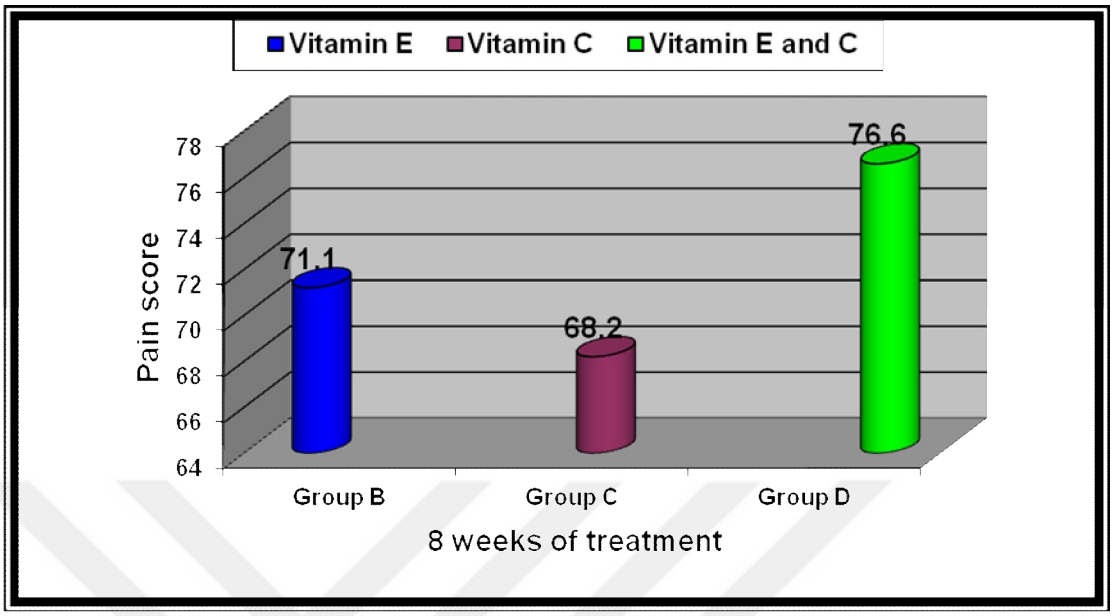


Figure (3.13): The effect of different antioxidant on pain score in OA patients treated with diclofenac sodium after 8 weeks of treatment.

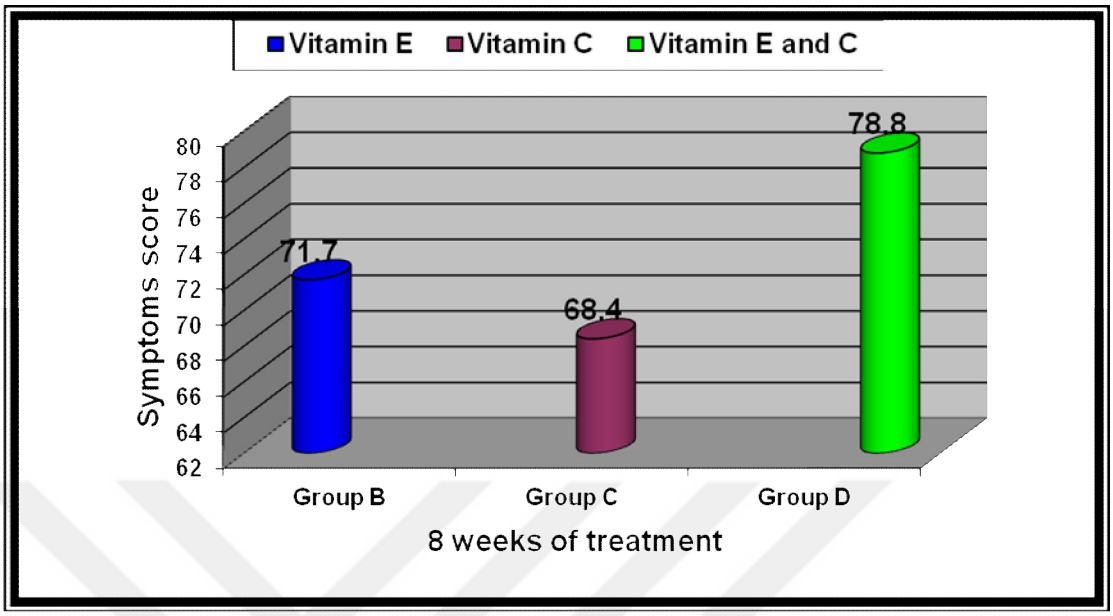


Figure (3.14): The effect of different antioxidant on pain score in OA patients treated with diclofenac sodium after 8 weeks of treatment

4. DISCUSSION

4.1 Correlation of oxidative stress with OA

OA is considered as the most common form of arthritis and the leading cause of disability [(129)]. It is account for more dependency in walking, stair climbing, and lower extremity task than any other disease with an impressive economic costs estimated as 3 times the cost of rheumatoid arthritis (RA). The risk of disability attributable to the knee OA alone is as great as that due to any other medical disorders in the elderly [(130)]. A recent world health organization report on the global burden of the disease indicates that OA is likely to become the fourth most important global cause of disability in women, and the 8 most important disease in men [(131)].

The typical patient with OA is middle aged or elderly complains of knee, hip, hand, or spine pain and stiffness in and around the affected joint, causing a decrease in the function of the joint and morbidity. The onset of these symptoms is mostly insidious, and pain typically worsens with the use of the affected joint, but usually is alleviated with rest [(132)].

The primary goals of OA treatment are to relieve pain, minimizing disability and limiting the progression of the disease. Because most patients with OA are elderly people who

have comorbidities with other disease and are more susceptible to the side effects of chronically used medications, care must be taken to individualize therapy on the basis of patient needs and to minimize potential drug toxicity. In spite of having various agents offering possibilities for pharmacological treatment of OA, among them are NSAIDs (selective and non-selective of COX-inhibitors), analgesics, and steroids [123], but the therapeutic outcomes, toxicity profile, and significant interference with the pathologic of the disease are not so much hopeful, and necessative search for new therapeutic models in this respect.

The normal cartilage has low level of Omega 6 which increases progressively with increasing age leading to increase in serum MDA level that pronounced in OA patients [133,134]. The endogenous defense mechanism against ROS is the intracellular antioxidant enzymes and micronutrient antioxidants that provide additional defense against tissue injury [135]. One of the methods for assessment the human defense mechanism against ROS is by measuring serum GSH level which found to be decreased in OA patients as compared with normal subjects due to defect in human defense mechanisms against ROS which can be used as indicating parameter for evaluating patient defense against ROS as used in this study, the increase in serum GSH level indicate the improvement of patient defense against ROS.

Table 3.1, 3.2, 3.6, 3.7 showed that, serum MDA levels were significantly ($p< 0.05$) increased while serum GSH levels were significantly decreased in OA patients as compared with the healthy control subjects which may be attributed to the over production of ROS, these results were consistent with that of Sowers et and Adkinsson et that one of the factors which contribute to the cartilage matrix degradation in OA is by reactive oxygen species ROS through lipid peroxidation by which oxidative decomposition of Omega-3 and Omega-6 polyunsaturated fatty acids (PUFA) of membrane phospholipids leading to formation of complex matrix of lipid hydroperoxide and aldehyde end products such as MDA [133, 134]. The decrease in human defense mechanisms against ROS (imbalance between ROS and antioxidants) can be seen through measurement of serum GSH level that significantly decreases as compared with the healthy control subjects. The maximum level of elevation in serum MDA was 90.5% in age intervals (40-49) years, and 82.5% in BMI intervals (19.1-25.8) kg/m^2, while the maximum level of reduction in serum GSH were (68.6%, 66%) in age intervals (40-49) years and BMI intervals (19.1-25.8) kg/m^2 respectively as compared with the healthy control subjects.

4.2 Correlation of oxidative stress with age in OA patients

The prevalence and incidence of OA in all joints are correlated with the age. Osteoarthritis has higher prevalence in women below 50 than in men over 50 [136]. The age related prevalence pattern are consistent with the role of postmenopausal estrogen deficiency in increasing the risk of OA

[137]. The increase in the incidence and prevalence of OA with age is more likely as a consequence of several biological changes that occur with aging such as decrease the responsiveness of chondrocyet to the growth factors that stimulates repairing properties and increasing in the laxity of ligaments around the joints making older joints unstable and susceptible to injury with gradual decrease in the strength and slowing of peripheral neurological responses which leading to the failure in shock absorbance and protectors of the joints [138].

The results of table 3.1, 3.6 were correlated with Sharma et al study that most OA patients were female over 50, the prevalence and incidence of OA were increased according to the age which may be due to the changes in lipid composition of the cartilage which associated with increased oxidative stress leading to the oxidation of cartilage collagen and lipid peroxidation [134,138].

4.3 Correlation of oxidative stress with weight in OA patients

Over weight persons often develop knee OA more than others [139,140]. Recent studies found that body mass index was directly and strongly correlated with the risk of developing knee OA [141,142]. Spector et a found that the obese women with unilateral disease were at higher risk of developing bilateral radiographic OA [143]. Felson et al reported that a weight loss of 11 pound (Ib) in women of medium height associated with 50% reduction in the risk of developing symptomatic knee OA [144]. Clinical trial of weight loss showed impressive correlation between the weight loss and the improvement in symptoms of

the knee OA [145].

Accordingly, data in table 2.7, 3.2, 3.7 indicate that most of OA patients were over weight which increases the amount of force across a weight bearing joint that induce cartilage breakdown [146] using BMI parameter when weight and height of each person were measured in order to obtain the load carried on hip, knee and lower extremity joints that increases as the weight increase and the height decrease, other pathway that increase the incidence of OA in obese persons is through metabolic intermediate [147,148].

Moreover table 3.2, 2.7 showed increasing in MDA level and decreasing in GSH level respectively as the BMI increase which consequently increases the incidence and progression of OA that related to Carman and Olivera studies et al(2008) respectively. Obesity is considered as strong risk factor in women with OA compared to men due to the excess of adipose tissue which might produce abnormal level of certain hormones or growth factors that affect cartilage or underlying bone [148].

4.4 Effect of antioxidants on oxidative stress in OA patients

Endogenous defense mechanisms against ROS that cause lipid peroxidation include intracellular antioxidants and micronutrients, thus supplementation of micronutrient antioxidants can provide additional defense against tissue injury and improve the imbalance between ROS and antioxidants [149,150]. Framingham and McAlindon studies showed that the high intakes of antioxidants were reduce the risk of cartilage loss and disease progression [151]. Antioxidant is important to

patient health given that ROS are a component of the processes that mediate tissue injury in OA patients [152].

Table 3.3, 3.8, 3.4, 3.9 showed increase GSH level and decrease MDA level with antioxidants intake indicate increase the defense mechanisms against oxidative stress and then lipid peroxidation after treatment with vitamin E and vitamin C in addition to that the improvement of symptoms and pain score as shown in table 3.13, 3.14, 3.15, 3.16, which consistent with a variety of studies demonstrated that antioxidants positively affect the process involved in cartilaginous cells and cartilage structure, therefore supplement of patients with micronutrient antioxidants (vitamin E and/or C) may increase chondrocyte repairing properties and then improving signs and symptoms in OA patients through clinical observation in addition to the laboratory investigation after 8 weeks of the treatment as compared with those OA patients before treatment (baseline) [153,154].

4.5 Effect of vitamin E on oxidative stress in OA patients

Vitamin E is the most abundant lipid soluble antioxidants in human lipoproteins and tissues that act as chain breaking antioxidants against lipid peroxidation [154]. The administration of vitamin E antioxidant in patients suffering from OA has been shown to enhance the reduction of joint pain, swelling and

tenderness [155].

Table 3.3 showed reduction in serum MDA level after 2, 4, 6, and 8 weeks of the treatment with diclofenac sodium, the maximum level of reduction was 6.5% after 8 weeks of the treatment as compared with the zero time (baseline), while in group B patients the maximum level of reduction in serum of MDA was 35.4% after 8 weeks of the treatment. Moreover the level of improvement in body defense mechanism against ROS indicated by high serum GSH level which increased by treating patients with diclofenac sodium with maximum level of elevation was 15.6% after 8 weeks of the treatment, compared to 152% for group B as shown in table 3.8 which consistent with Parker and Edmonds studies et al respectively were studying the effect of vitamin E antioxidant on OA patients.

Concerning symptoms and pain score outcome table 3.13, 3.14 were showed that using of diclofenac sodium alone improve symptoms and pain score in maximum level of (19.1%, and 30%) respectively after 8 weeks of the treatment while the maximum improvement in symptoms and pain score was (35.2%, and 37.9%) respectively in group B after 8 weeks of the treatment which consistent with Edmonds and Witten et al respectively [155,156].

4.6 Effect of vitamin C on oxidative stress in OA patients

Vitamin C acts as an antioxidant through its electron donor property (as reducing agent) to free radicals and reactive oxygen species [157], Kraus et al (2004) showed that the low dose of vitamin C represented the minimum amount needed to prevent

scurvy, the medium dose of vitamin C which is equivalent to the plasma level comparable with those achieved in persons consuming 200 mg/day, and high dose of vitamin C supplementation increase cartilage collagen content [158].

This study was correlated with the Framingham study of knee OA which showed that the population with medium to higher intake of vitamin C had a reduced risk of progression of radiographic knee OA, while persons in the lowest tertile of vitamin C intake had 3-fold greater risk of progression of knee OA, joint space loss, and even onset of knee pain compared with those with higher intake [151].

Table 3.4 showed that, vitamin C having antioxidants activity in OA patients by reducing serum MDA level and improvement serum GSH level, were the maximum level of reduction in serum MDA was 24% after 8 weeks of the treatment for group C compared to 6.5% reduction in serum MDA level for group A as compared with the baseline, these results will indicate vitamin C have lipid peroxidation chain breaking properties that decreases MDA level in OA patient and then progression of disease in table 3.9 the maximum level of elevation in serum GSH was 123% after 8 weeks of the treatment for group C, compared to 15.6% for group A as compared with the baseline, therefore vitamin C improve human defense against ROS in addition to improving in serum GSH level table 3.15, 3.16 indicate that the addition of vitamin C to diclofenac sodium regimen increase symptoms and pain score and the maximum level was (32%, and 35%) respectively after 8

week of the treatment compared to (19.1%, and 30%) improvement in symptom and pain score respectively in patient group treated with diclofenac sodium alone as compared with the baseline, these results approve that the addition of antioxidant to OA treatment regimen will slow the progression of disease and improve patient outcome score with minimum side effect and cost compared with other types of the treatment.

Table 3.5, 3.10 showed that, the use of combined antioxidant (vitamin E plus vitamin C) in OA patients had synergistic effect compared with the use of single antioxidant (vitamin E or vitamin C), the serum level of MDA was 37.6% in patients treated with diclofenac sodium plus vitamin E and C, compared to 6.5% for patient treated with diclofenac sodium alone after 8 weeks of the treatment as compared with baseline (zero time), in addition to that GSH level was 223% in group D that treated with combined antioxidant compared to 15.6% in group A treated with diclofenac sodium alone.

Table 3.3, 3.4 showed that, serum MDA level reduced when single antioxidant vitamin E or vitamin C added, the maximum reduction level was 35.4% in group B patients on vitamin E, and 24% in group C patients on vitamin C compared with 37.6% reduction when both antioxidants combined at end of 8 weeks of the treatment (table 3.5), likewise table 3.8, 3.9 showed that GSH level was (152%, 123%) for group B on vitamin E and group C on vitamin C respectively, compared with results in table 3.10 when vitamin E and C given together (group D) elevation in serum GSH level was 223% as compared

with the baseline.

4.7 Antioxidants and pain score in OA

OA is the single most common cause of disability in older, many studies about the incidence and prevalence of knee pain, disability and radiographic changes during OA in the general population revealed 10% prevalence of painful disabling knee OA in people over 55 years of when one quarter are severely disabled. Taking into account the assumption that if the pathological process which gives rise to X-ray changes could be slowed down this would be an important mean of preventing pain and disability and improve Symptoms score in OA patients, especially those who present with knee pain, morning stiffness, and joint crepitus according to reported guideline published by American of rheumatology about classification of OA as a clinical symptom in older adults [(159)].

In this respect treatment with NSAIDs alone or in combination with antioxidants results in significant elevation in symptoms score compared to baseline values, but the increase in symptoms score in the patient group treated with NSAIDs alone was significantly lower than those in groups of patients treated with NSAIDs plus antioxidants. This results may give promising indication about the possible role of antioxidants which display powerful regenerating and chondrocyte protective properties at least retarding the progression of degeneration process which predispose to pain and consequent disabling and other symptoms in OA.

In symptoms and pain score the use of combination of vitamin E and vitamin C results in synergistic effect which indicated in table 3.17, 3.18 that the percent of improvement symptoms and pain score were (41.2%, and 45%) respectively compared to (35.2%, and 37.9%) for patients on vitamin E alone table 3.13, 3.14, while the percent of improvement in patient group on vitamin C was (32%, and 35%) for symptoms and pain score respectively as shown in table 3.15, 3.16, therefore using of combined antioxidants in OA patient may improve patient outcome and compliance and slow the progression and destruction of cartilage matrix more than that achieved when single antioxidant used, but remain with good outcome than that if no antioxidant is used.

Conclusion

There was a significant role for oxidative stress in the pathogenesis and progression of OA through elevation of serum MDA level and reduction of serum GSH level. There was a significant role for oxidative stress in the pathogenesis and progression of OA through reduction of symptoms and pain score. Vitamin E and C in a dose of 400 mg and 500 mg twice daily respectively for 8 weeks showed effect on lowering serum MDA level and elevating serum GSH level with significant improvement in symptoms and pain score. The combination of vitamin E and C in a dose of 400 mg and 500 mg twice daily respectively were preferable than using single antioxidant in OA patients treated with diclofenac sodium .All that data result which I got it , negative with African races so I neglected from my data sheet and didn't counted because they didn't get any improvement in their clinical conditions.

Recommendation for further studies

1. To determine the possible effects of vitamin E or C on other types of

Disease

2. To determine the effects of vitamin E or C on other investigation

parameters which might effect patient outcome score.