Original article:

A comparative study between Antioxidant vitamin and Kellgren–Lawrence grading scale in knee osteoarthritis patients

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Abstract:

Osteoarthritis (OA) is a strongly age-related joint disorder that is defined as a gradual loss of articular cartilage. Current concepts of the pathogenic mechanisms of OA suggest that there isa shift in the homeostatic balance between the destruction and synthesis of bone and cartilage,with a net progressive destruction of these tissues. Recent studies of the biology of chondrocytes show that these cells actively produce reactive oxygen species (ROS). ROS are capable of inducing apoptotic cell death in chondrocytes, but more important, they can result in the degradation of aggrecan and collagen. Micronutrient antioxidants such as alpha-Tocopherol (Vitamin E) and Vitamin C also act to reduce the effects of ROS. A hospital based case control study was conducted in Burdwan Medical College, West Bengal, India on 76 post-menopausal women aged 45–70 years suffering from Osteoarthritis (OA) of the knee joint, which was diagnosed from symptoms, clinical examinations and radiographic findings. The 151 controls were selected from the healthy persons .plasma vitamin C and serum vitamin E ware measured.. Knee joint radiographs were evaluated with the Kellgren–Lawrence grading scale .Furthermore both were correlated with Kellgren–Lawrence grading scale to find out possible association between the antioxidant vitamins and the disease progression. Results showed that there was a significant decrease in antioxidant vitamin levels in patients with knee joint osteoarthritis as compared to the controls suggests that treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy. Both antioxidant vitamins showed negative correlation with Kellgren–Lawrence grading. Both vitamins decreased with increasing Kellgren–Lawrence grade. So antioxidant vitamin can be use to assess the disease severity.

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Introduction:

Osteoarthritis (OA) is a strongly age-related joint disorder that is defined as a gradual loss of articular cartilage, combined with thickening of the subchondral bone, bony outgrowth at joint margins, and mild, chronic nonspecific synovial inflammation.[1]The most common parts in the knee to be affected are the medial tibiofemoral and lateral patellofemoral compartments. The clinical features of OA include pain, short-lasting stiffness, cracking of joints, joint swelling, and limited range of motion. Diagnosis of OA is dependent upon history and physical examination in conjunction with radiographs demonstrating joint space narrowing, osteophyte formation, and subchondral bone sclerosis.

Current concepts of the pathogenic mechanisms of OA suggest that there is a shift in the homeostatic balance between the destruction and synthesis of bone and cartilage, with a net progressive destruction of these tissues[2]. The destructive process is mediated by the production of aggrecanases and matrix metalloproteases that selectively degrade cartilage matrix[3-5]. In addition to the role of proteases in matrix destruction, recent studies of the biology of chondrocytes show that these cells actively produce reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, hydroxyl radicals, and nitric oxide[6-12] . ROS are capable of inducing apoptotic cell death in chondrocytes, but more important, they can result in the degradation of aggrecan and collagen[13-18].

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Moreover, the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions. [19]. Antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated. [20]. They exist in both the aqueous and membrane compartment of cells and can be enzyme or not enzyme.

Micronutrient antioxidants such as alpha-Tocopherol (Vitamin E), ?-carotene and other carotenoids, and Vitamin C also act to reduce the effects of ROS. In related experiments, guinea pigs supplemented with Vitamin C in drinking water were protected from experimentally-induced cartilage degeneration of the knee [21]. Ingestion of Vitamin E has also been shown to preserve cartilage in animals [22]

However, few studies have been done to establish the relationship between antioxidant vitamins and clinical severity of disease. Aim of this study was to analyze the relationship between antioxidant vitamins and the clinical severity of knee joint osteoarthritis. . The severity of OA was graded by the Kellgren–Lawrence grading scale [23]which is as follows:

Grade 1: doubtful narrowing of joint space and possible osteophytic lipping;

Grade 2: definite osteophytes and possible narrowing of joint space;

Grade 3: moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour;

Grade 4: large osteophytes marked narrowing of joint space, severe sclerosis and definite deformity of bone contour.

Ascorbic acid and plasma vitamin E were measured to assess the antioxidant vitamin parameters.

Vitamin C readily scavenges the ROS, $ONOO^-$, NO_2, NO and HOCl[24]. Although Vitamin C reacts rapidly with $OH^\bullet$ (rate constant $>10^9$L/mole/sec Vitamin C may prevent the formation of $ONOO^-$ by reaction with $O_2^{\bullet-}$ and may help to release NO from endothelial cells by preventing the oxidation of LDL[25]. Vitamin C can also act as a co-antioxidant by regenerating alpha tocopherols from alpha-tocopheroxyl radical produced during scavenging of ROMs[26]

The major function of vitamin E is to work as a chain-breaking antioxidant in a fat soluble environment thus preventing the propagation of free radical reactions. Vitamin E is a lipid radical scavenger and especially protects polyunsaturated fatty acids (PUFA) within membrane phospholipids and in plasma lipoproteins. Vitamin E provides easily donated hydrogen to the lipid reaction and an antioxidant radical is create .Then, the new antioxidant radical combines with other antioxidant radicals and becomes harmless or combines with ascorbic acid and is converted back to ? tocopherol.

Methods

The present hospital based case control study was undertaken in the Department of Biochemistry with collaboration of the Department of Orthopedic, Burdwan Medical College and Hospital, West Bengal. The cases were selected from the patients attending the outdoor of Orthopedic Department of Burdwan Medical College and Hospital.

Subjects

The present study is based on 76 postmenopausal women aged 45–70 years (mean of 54.5) with OA of the knee, which was diagnosed from clinical symptoms, examinations and radiographic findings. Secondary OA patients, such as post-traumatic OA cases, were excluded from the study. **.** The 151 controls were selected from the healthy persons who accompanied the patients from same region. The controls were not suffering from any chronic or metabolic disorder at the time of diagnosis**.** All patients fulfilled the ACR criteria for knee OA [27]. The procedures followed were in accordance with the principles of the Declaration of Helsinki in 1975, as revised in 1983.

Knee joint radiographs

Antero-posterior weight-bearing radiographs of both knees were taken. The bilateral weight-bearing antero-posterior knee radiograph was taken with the patient standing with toes pointed straight ahead, knees fully extended, and weight equally distributed on both feet. The Kellgren–Lawrence grading of radiographs was done by a radiologist who was blinded to the source of subjects. The joint space width of the medial and lateral compartments of the knee joint was measured in millimeters. A vertical

line was drawn from the midfemoral medial and lateral condyles to the tibial plateau, and the lesser of the two measurements was taken as the joint space width [28]

Serum ?- tocopherols estimation [29]

Estimation of tocopherol in serum has been done by Baker and Flank method in 1968. Serum tocopherols can be measured by their reduction of ferric to ferrous ions which then form a red colour complex with ?-dipiridyl. Tocopherols being lipid soluble are first extracted into xylene and the absorbance is read at 460nm[257]

Determination of plasma ascorbic acid (Photometric method)

Ascorbic acid in plasma is oxidised by Cu^{2+} to form dehydroascorbic acid, which reacts with 2, 4 dinitrophenylhydrazine to form a red bis-hydrazone, which is measured at 520nm[30].

Specimen

Venous blood was collected from all of the above persons from the median anticubital vein. The blood from each person was divided into two aliquots as follows:

Heparinised blood for assessment of plasma ascorbate ,

Clotted blood for assessment of serum alpha tocopherol level.

Result

The obtained data was analyzed using SPSS software. The Table 1 displayed the comparison of mean of different antioxidant vitamin parameters between cases and controls**.** Antioxidant vitamins plasma ascorbate and serum alpha tocopherol showed significantly lower values in cases. This table 2 shows a significant positive correlation between plasma vitamin C and serum vitamin EFrom the table 3 it is evident that radiological grading has a strong negative correlation with both plasma vitamin C and serum vitamin E.ANOVA of the plasma vitamin C and serum vitamin E of the various groups of subjects categorized clinically as per the Kellgren –Lawrence grading scale shows there is significant difference between them(table 4) The relationship between the Kellgren–Lawrence grading and the plasma vitamin C is shown in box whisker plot (fig 1). Here the plasma vitamin C decreases with increasing Kellgren–Lawrence grade. Vitamin C showed variances of 0.011, 0.006, 0.002, 0.002 and skewness of 0.603, 0.907, -0.054, -0.463 for grade 1, grade 2, grade3 and grade 4 respectively. The relationship between the Kellgren–Lawrence grading and the serum vitamin E is shown in box whisker plot (fig 2). Here the serum vitamin E decreases with increasing Kellgren–Lawrence grade. Vitamin E showed variances of 0.157, 0.034, 0.029, 0.028 and skewness of 0.082, 0.493, 0.58, -0.132 for grade 1, grade 2, grade3 and grade 4 respectively.

Discussion

To scrutinize our findings related to the possible imbalance in the redox status estimations of plasma vitamin C and serum vitamin E were performed to assess the overall antioxidant status of individual.

Table1 showed observed that there is a significant decrease in the levels plasma ascorbic acid and serum vitamin E (non-enzymatic antioxidant defense system) in patients with osteoarthritis when compared to controls. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to the increased turnover for preventing oxidative damage in these patients, suggesting an increased defense against oxidant damage in osteoarthritis.

The antioxidant vitamin, vitamin C and vitamin E are positive correlated with each other (table 2). These findings suggested that for maintenance of the normal level of alpha tocopherol a normal level of plasma ascorbate was also necessary. These findings correlate well with the fact that in lipid peroxidation, when alpha tocopherol attenuates the process by breaking the chain of free radical propagation, ascorbic acid helps in the regeneration of the alpha tocopherol activity[31]

The severity of the disease process evaluated with the help of Kellgren–Lawrence radiological grading.

Antioxidant vitamins plasma vitamin C and serum vitamin E showed a significant strong negative correlation radiological grading (Table 3). Both ware decrease with increasing Kellgren–Lawrence grade. (Box whisker plot –fig-1 and 2).

ANOVA of the plasma vitamin C and serum vitamin E of the various groups of subjects categorized clinically as per the Kellgren –Lawrence grading scale also shows there is significant difference between them(table 4)

All data signified that with increasing disease severity of osteoarthritis there was simultaneous decrease in antioxidant vitamins. It may be due to increase consumption of antioxidant vitamin with increase ROS mediated tissue damage. ROS contribute to cartilage matrix degradation in OA. . Micronutrient antioxidants such as alpha-Tocopherol (Vitamin E), ?-carotene and other carotenoids, and Vitamin C act to reduce the effects of ROS. In related experiments, guinea pigs supplemented with Vitamin C in drinking water were protected from experimentally-induced cartilage degeneration of the knee [32]. Ingestion of Vitamin E has also been shown to preserve cartilage in animals [33]

In human subjects, dietary intake of vitamin E and zinc, well below recommended dietary amounts, caused an increase in severity of rheumatoid symptoms [34]In the Framingham Osteoarthritis Cohort Study, high intake of antioxidants reduced the risk of cartilage loss and disease progression, though there was no association with incidence of OA [35]. Several studies in humans have suggested that vitamin E was beneficial for treatment of human OA Vitamin C appeared to improve symptom [36]. Vitamin E is lipophilic compared to vitamin C which is hydrophilic. Vitamin E's solubility and uptake in the body may be reduced in comparison to vitamin C due to its hydrophobic character. Finally, vitamin E is about five times weaker in terms of its ability to soak up ROS compared to vitamin C.

Conclusion:

The present study conducted an analysis on antioxidant vitamin status in the osteoarthritis patient's and how it related with disease severity. Antioxidant vitamins were significantly lower in the osteoarthritis patients as compared with the control population as indicated by the plasma vitamin C and serum vitamin E. The study, hence, suggests that treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage and deterioration of the musculoskeletal tissues in osteoarthritis. But further multicentre placebo controlled trials are needed to establish it. There was a significant association between the antioxidant vitamins and the severity of the disease process as indicated by the radiological grading which may help in understanding the biochemical basis and etiopathologic mechanisms of the disease process. So antioxidant vitamin can be use to assess the disease severity of osteoarthritis patient but further detailed and more comprehensive studies designed on a longitudinal basis are much needed.

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