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A PROSPECTIVE DOUBLE BLIND COMPARATIVE STUDY OF MEDICATIONS PRESCRIBED FOR THE TREATMENT OF PATIENTS WITH KNEE OSTEOARTHRITIS

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ABSTRACT **INTRODUCTION** Knee Osteoarthritis (KOA), is a degenerative joint disease, which is extremely common in persons over 35 yrs of age and is one of the most prevalent diseases of elderly people. KOA breaks down the cartilage in our joints which when affected our bones rub together causing pain, swelling and loss of motion of joint. There are 'n' numbers of combinations are being used for therapy of KOA. In our study, we have chosen GlcN1 (glucosamine sulfate with potassium chloride), GlcN2 (glucosamine sulfate plus chondroitin sulfate) along with ChoN3 (chondroitin sulfate alone)] in the treatment of knee osteoarthritis. **Methods:** A total number of 135 patients with KOA were divided into three groups, first group received GlcN1 500 mg of glucosamine sulfate with potassium chloride one capsule thrice daily for 12 weeks. Second group received GlcN2, containing glucosamine sulfate 500 mg plus chondroitin sulfate 400 mg, 2 capsules thrice daily for 12 weeks. Third group received ChoN3, chondroitin sulfate 400 mg, methyl sulfonyl methane 250 mg and manganese 20 mg, one tablet thrice daily for 12 weeks. In this study, we observed correlation of measurement of urinary pyridinium cross links such as pyridinoline (Pyr) and deoxypyridinoline (Dpyr). **Results:** In this study, WOMAC scores pertaining to daily activities were compared with different time intervals as well as between treatment groups. In GlcN2 and ChoN3 group the difference in WOMAC daily activity scores was significantly ($P<0.001$) decreasing at the end of 4,8,12 and 16 weeks of therapy, compared to base line. In over all the WOMAC score of pain, stiffness and difficulty in performing daily activities was retained significantly ($P<0.001$) at the end of 16 weeks even after stoppage of drug therapy. The level of Pyr excretion in urine was significantly ($P<0.001$) diminishing in GlcN2 and ChoN3 group when compared to GlcN1 group at different time intervals (after 8, 12 and 16 weeks) even after stoppage of drug treatment. **Conclusion:** The present study concludes that GlcN2 and ChoN3 have superior efficacy and safety in view of relieve pain, improving functional ability and joint mobility so as to enhance the quality of life for KOA patients rather than GlcN1.

KEYWORDS : : Double Blind, Glucosamine & Chondroitin sulfate, Pyridinium Crosslinks, Bone Resorption Analysis.

Introduction:

Osteoarthritis (OA) of knee is a major problem persisting all over the world prevailing in old age people; particularly in women, and is a common, chronic, progressive, skeletal, degenerative disorder [1,2]. The pharmacological management of OA is largely palliative, focusing on alleviation of symptoms. The present scenerio recommendations for the management of OA include a combination of non-pharmacological interventions (weight loss, education programmes, exercise, continued physiotherapy, etc.,) and pharmacological treatments such as non-steroidal anti-inflammatory drugs (NSAIDs), etc.,[3]. The degenerative disorder of OA is mainly affects the mobility of individuals and their quality of life [4]. The NSAIDs mainly act by blocking prostaglandin synthesis. The other mediators of inflammation like leukotrienes and complement pathways are not influenced by NSAIDs, which is associated with severe side effects (dyspepsia, upper abdominal pain, gastrointestinal bleeding) following medium to long term administration [4-6] and also do not reverse the pathological process of the disease. In this context, there is a need for safe and effective alternative treatment while the absence of any cure reinforces the importance of prevention. Such prevention as well as alternative treatments could achieve in form of nutrition. It is now increasingly recognized that, beyond meeting basic nutritional needs, nutrition may play a beneficial role in some diseases [7].

Glucosamine and chondroitin sulfate are two commonly used nutraceutical compounds that have been reported to have chondroprotective properties [8]. Glucosamine is a hexosamine sugar and a basic building block for the biosynthesis of the glycosam inoglycans (GAG), and proteoglycans that are important constituents of the articular cartilage. Chondroitin is a polymer of the repeating disaccharide unit of galactosamine sulfate and glucuronic acid, which is one of the predominant glycosaminoglycan that is found in the proteoglycans of articular cartilage. Both are animal products having anti-arthritic and anti-inflammatory activities.[9,10] Earlier reported study shows that both glucosamine and chondroitin have potential in the treatment of OA even if they show moderate efficacy [11,12]. These compounds have been used for OA in Europe and USA for more than a decade and recently have acquired substantial popularity. A meta analysis by McAlindon and coworkers demonstrated improvement of pain in patients with OA [13,14]. The efficacy of oral glucosamine sulfate (GSO_4) 1500 mg (500 mg three times daily) in the treatment of OA have demonstrated to decrease in joint pain, tenderness, swelling and an increase in joint mobility with well tolerated [15,16].

Urinary pyridinium crosslinks such as pyridinoline (Pyr) and deoxypyridinoline (Dpyr) measurement are used to monitor the clinical status as well as bone turnover of OA patients. These two collagen crosslinks measured in urine, which provides information both on the pathogenesis of OA as well as the rate of bone turnover. Because pyridinium crosslinks are found extensively in bone cartilage, it is excreted in urine in higher amounts when cartilage breaks down. For this reason research indicates that it may serve as an important biomarker for assessing joint destruction in OA [17, 18]. So far, no comparative study was noticed with wide range of patient population with glucosamine [GlcN1 (glucosamine sulfate with potassium chloride), GlcN2 (glucosamine sulfate plus chondroitin sulfate) along with ChoN3 (chondroitin sulfate alone)] treated group with correlation of measurement of urinary pyridinium cross links such as Pyr and Dpyr. Hence, this study was planned to evaluate the efficacy, safety and tolerability of glucosamine with chondroitin sulfate treated groups.

Patients and Methods:

Study Design:

Double blind prospective randomized comparative trial. Informed written consent was obtained from all 135 patients. The study design, population, intervention and outcome measures were based on CONSORT guidelines [19].

Sample Size:

135 patients aged between 40 and 69 years of either gender with primary OA of knee, diagnosed according to the criteria of American College of Rheumatology [20] were enrolled from outpatient department of Sudha Institute of Medical Sciences, Erode, Tamilnadu, India.

Study Period:

The study was conducted over period of 8 months from January 2014 to August 2014.

Study Site:

The study was carried out in outpatient department of Sudha Institute of Medical Sciences, Erode- 638011, Tamilnadu, India.

Inclusion Criteria:

1. Knee OA patients, either sex with symptoms of OA,
2. Age – 40 to 69 years,
3. Clinical presentation and X-ray verified reduction in interarticular space were considered,
4. Complete clinical evaluation plus hemogram, liver and renal biochemistry were done in all cases that were on KOA.

Exclusion Criteria:

1. Patients with current or recent (less than two weeks) anti-rheumatic therapy
2. Patients having arthrosis secondary to systemic disease,
3. Patients having suspected bacterial infection of the joint,
4. Patients existing pregnancy and lactation,
5. Patients with known hypersensitivity to active principles or auxillary substances of test drugs,
6. Patients with hemorrhagic disorders, history of peptic ulcer, acid peptic disease, concurrent illness, receiving concomitant drug therapy,
7. History of drug allergy and who had undergone corticosteroid therapy in the last two months were also excluded.

Ethical Consideration:

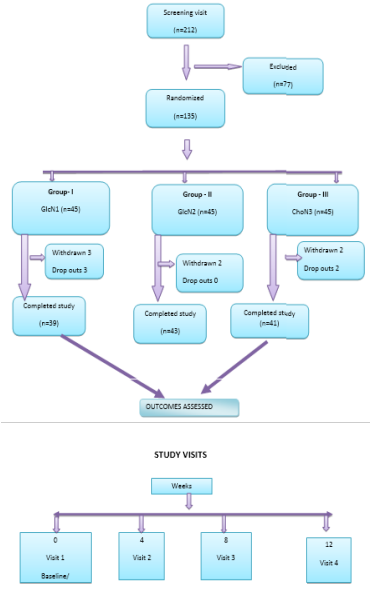
All the investigational procedures and protocols used in this study were reviewed & approved by the Institutional Ethical Committee (IEC Reference No: 02/2014) and were in accordance with the CONSORT guidelines.

Study Intervention:

135 KOA patients randomly allocated (randomization done by SAS system for windows) into three groups of 45 patients in each group. First group received GlcN1 (Cap. Cartigen, Manufactured by Pharmed, Bangalore, India, containing 500 mg of glucosamine sulfate with potassium chloride per capsule) one capsule thrice daily for 12 weeks. Second group received GlcN2 (Cap. Rejoint, manufactured by Nicholas Piramal Ltd, Mumbai, India, containing glucosamine sulfate 500 mg plus chondroitin sulfate 400 mg present in each yellow and blue capsule respectively) one yellow and blue capsule thrice daily for 12 weeks. Third group received ChoN3 (Tab. Conjoint, manufactured by Medley Pharmaceuticals Ltd, Mumbai, India, containing chondroitin sulfate 400 mg, methyl sulfonyl methane 250 mg and manganese 20 mg) patients were advised to take only pink colour tablet thrice daily for 12 weeks. The medicines were given orally and the patients administered the medications after meals. Consumption of 90 % of the drug was considered as adequate compliance. The clinical orthopaedic investigator, radiologist and patients were blind about the intervention and medications. Basically all the medications were transferred to separate plastic cover which provided with numerical numbering and bar coded according to treatments groups. . Figure 1. showing the disposition of patients by flow chart method.

Figure 1. Flow chart showing the disposition of patients

TRIAL FLOW CHART



Study Evaluation:

The patients were assessed by Western Ontario and McMaster Universities OA index (WOMAC) scale (version VA 3.1) at base line and at the end of 4, 8 , 12 and 16 weeks respectively. This is a modified visual analogue scale which consists of questions based on 3 symptoms i.e pain, stiffness and difficulty in performing daily routine physical activity. For each question the patient has to mark on scale between 0 and 100. Score 0 indicates no pain and maximum score 100 is given for severe pain. Adding up the scores of all the questions for a particular symptom gives total score for that symptom. Decrease in score suggests symptomatic improvement [21]. An Anterio-posterior radiograph of the affected knee joint was done at base line and after 12 weeks of therapy. The radiographs verified for joint space narrowing and graded according to Kellegren and Lawrence's criteria [22,23] as specified in Table 1. The patients were permitted to continue physiotherapy as per the recommendation of the orthopaedician and advised to report ADRs. Routine hematological investigations were done on all patients. The observations were decoded, tabulated and then analyzed.

Table. 1. Radiological scoring for knee osteoarthritis [22,23].

Radiological Scoring	X-ray findings
0	Normal; no changes.
1	Doubtful joint space narrowing.
2	Minimal change, mostly characterized by osteophytes.
3	Moderate change, characterized by multiple osteophytes and/or definite joint space narrowing.
4	Severe change, characterized by marked joint space narrowing with bone on bone contact with large osteophytes.

Bone resorption assessment

This is a convenient non-invasive method, first morning void urine samples were colleted from the patients at base line and at the end of 8, 12 and 16 weeks respectively. Aliquots of urine with no preservatives added were stored at -20ºC until analysis. The urinary Pyr and Dpyr levels were measured by a high performance liquid chromatography (HPLC) according to Eyre *et al* [24] and followed by some modifications done by Marowska *et al* method [25]. The technician who carried out urine analysis was blinded observer only.

Power calculation

Patients' numbers were calculated to detect a 5-point difference in improvement in WOMAC scale between groups at a 5% significance level with 80% power.

Statistical analysis

The data is represented as mean ± SEM. Statistically significant difference was ascertained by '*P*' value which is considered significant of $P<0.05$ and highly significant $P<0.01$ and $P<0.001$ as comparisons of different groups patients were done using repeated measures of one-way ANOVA followed by Dunnett's multiple comparison test. Statistical analysis was carried out with GraphPad InStat Version 3 (GraphPad Software Inc., Camino Real, San Digeo, USA).

Observation and Results:

Out of 212 screened patients, 77 were excluded and 135 were enrolled based on the inclusion and exclusion criteria. The reasons for exclusion were:

a. Patients with current or recent (less than two weeks) anti-rheumatic therapy (28)
b. History of drug allergy and who had undergone corticosteroid therapy in the last two months (16)
c. Patients with hemorrhagic disorders, history of peptic ulcer, acid peptic disease, concurrent illness, receiving concomitant drug therapy (33)

The 135 recruited patients were randomized into 3 groups I, II and III consisting of 45 patients each. Patients of group I received GlcN1 (Cap. Cartigen, Manufactured by Pharmed, Bangalore, India, containing 500 mg of glucosamine sulfate with potassium chloride per capsule) one capsule thrice daily for 12 weeks, group II received GlcN2 (Cap. Rejoint, manufactured by Nicholas Piramal Ltd, Mumbai, India, containing glucosamine sulfate 500 mg plus chondroitin sulfate 400 mg present in each yellow and blue capsule

respectively) one yellow and blue capsule thrice daily for 12 weeks and group III received ChoN3 (Tab. Conjoint, manufactured by Medley Pharmaceuticals Ltd, Mumbai, India, containing chondroitin sulfate 400 mg, methyl sulfonyl methane 250 mg and manganese 20 mg) patients were advised to take only pink colour tablet thrice daily for 12 weeks. In Group I- three patients were withdrawn from the study due to 2 patients had other health related complications with standard therapy which required additional care and therapy and 1 patient had abnormal laboratory parameters. In Group II- two patients were withdrawn from the study due to 2 patients had developed with symptoms of jaundice which requiring additional care and therapy. In Group III- two patients were withdrawn from the study due to kidney stone problem which requiring additional care and therapy. In group I and III, 3 and 2 patients respectively, who were failed to follow up due to personal reason. At the out set, In Group I,II and III, 39, 43 and 41 patients respectively who were completed the study. The results at the end of the study are detailed. The medicines were given orally and the patients administered the medications after meals. Consumption of 90 % of the drug was considered as adequate compliance. The clinical orthopaedic investigator, radiologist and patients blind about the intervention and medication. Basically all the medications were transferred to separate plastic cover which provided with numerical numbering and bar coded according to treatments groups.

In our double blind clinical study, we compared the efficacy of salt forms of glucosamine containing preparations (GlcN1, GlcN2 and ChoN3) with chondroitin sulfate alone treated group in KOA patients. Of 135 patients enrolled in the study, 45 patients (13 men; mean age 59 years) received GlcN1, 45 patients (11 men; mean age 57 years) received GlcN2, and 45 patients (9 men; mean age 58 years) received ChoN3. All the patients showed narrowing of joint space on radiograph. Patients comparable with respect to their demographic features to all three groups (Table 2). 89 % patients showed the compliance to the study. The basal WOMAC scores for pain were compared with the scores after 4,8,12 and 16 weeks of therapy; as well as which is compared with three treatment groups (Table 4). In GlcN2 group the difference in WOMAC pain scores was significantly (P<0.01 and P<0.001) decreasing at the end of 4,8,12 weeks and even 4 weeks after stoppage of therapy when compared to GlcN1 treated group. Simultaneously GlcN1 group shown decreasing in WOMAC pain score at 4,8 and 12 weeks of therapy. At the same time WOMAC pain scores was high in GlcN1 group when compared to GlcN2 group at the end of 16th week.

The stiffness scores were assessed to all treatment groups and compared with different time intervals as well as between treatment groups (Table 5). In GlcN2 and ChoN3 group the difference in WOMAC stiffness scores was significantly (P<0.001) decreasing at the end of 4 and 16 weeks when compared to GlcN1 group. There was no specific difference observed in between GlcN2 and ChoN3 treated group in the aspect of stiffness score.

Table. 2. General characteristics of study group.

Number of patients recruited	135
Number of patients in group I (GlcN1)	**45**
Number of patients in group II (GlcN2)	**45**
Number of patients in group III (ChoN3)	**45**
Number of patients completed the study	**123**
Age	55 ± 5.02 (Yr., Mean ± SD)
Sex (M: F)	33:72
Body weight	70.7 ± 4.5 (Kg., Mean ± SD)
Height	159 ± 10.12 (Cm, Mean ± SD)
Patient compliance	Good

Table. 3. Radiological scoring of knee OA for different treatment groups.

Time	GlcN1 (n=45)	GlcN2 (n=45)	ChoN3 (n=45)
Base line	2.50 ± 0.13	2.21 ± 0.19	2.18 ± 0.15
After 12th week	2.34 ± 0.18	1.86 ± 0.17	1.78 ± 0.10

Values are in mean ± SEM (n=45). No significance difference was observed in between base line and after 12th week of therapy. (Repeated measure of one-way ANOVA followed by Dunnett's multiple comparison tests)

Table. 4. Pain scores of GlcN1, GlcN2 and ChoN3 treated OA knee patients at different time intervals.

Time	GlcN1 (n=45)	GlcN2(n=45)	ChoN3 (n=45)
Base line	243.9 ± 7.1	247.4 ± 9.16	239.4 ± 8.01
After 4 Weeks	208.1 ± 5.14##	202.89 ± 8.45***,###	205.9 ± 6.45***,##
After 8 Weeks	130.4 ± 7.4###	121.45 ± 10.2***,###	125.6 ± 4.1***,###
After 12 Weeks	73.4 ± 6.3###	62.78 ± 6.5**,###	60.1 ± 11.3**,###
After 16 Weeks	65.85 ± 5.14###	63.25 ± 5.45***,###	64.8 ± 8.45***,###

Values are in mean ± SEM (n=45); **P<0.01, ***P<0.001 Vs GlcN1 treated group; #P<0.05, ##P<0.01, ###P<0.001 Vs Base line. (Repeated measure of one-way ANOVA followed by Dunnett's multiple comparison tests)

Table. 5. Stiffness scores of GlcN1, GlcN2 and ChoN3 treated OA knee patients at different time intervals.

Time	GlcN1 (n=45)	GlcN2(n=45)	ChoN3 (n=45)
Base line	88.7 ± 4	84.4 ± 7.02	81.68 ± 3.2
After 4 Weeks	61.7 ± 8.2##	59.53 ± 5.2***,##	57.6 ± 4.3***,##
After 8 Weeks	46.9 ± 7.59##	44.56 ± 4.8##	43.5 ± 6.78##
After 12 Weeks	38.8 ± 3.35##	28.5 ± 1.8**,##	26.6 ± 4.3**,##
After 16 Weeks	37.2 ± 8.4##	23.5 ± 1.8**,##	24.2 ± 4.3***,##

Values are in mean ± SEM (n=45); **P<0.01, ***P<0.001 Vs GlcN1 treated group, ###P<0.001 Vs Base line. (Repeated measure of one-way ANOVA followed by Dunnett's multiple comparison tests)

WOMAC scores pertaining to daily activities were observed for all treatment groups and compared with different time intervals as well as between treatment groups (Table 6). In GlcN2 and ChoN3 group the difference in WOMAC daily activity scores was significantly (P<0.001) decreasing at the end of 4,8,12 and 16 weeks of therapy and even 4 weeks after stoppage of therapy when compared to base line. In over all the WOMAC score of pain, stiffness and difficulty in performing daily activities was retained significantly (P<0.001) at the end of 16 weeks even after stoppage of drug therapy.

The pyridinoline (Pyr) and deoxypyridinoline (Dpyr) levels were measured using the HPLC and compared with three treatment groups at different time intervals as well as between treatment groups (Table 7 and Table 8 respectively). The level of Pyr excretion in urine was significantly (P<0.001) diminishing in GlcN2 and ChoN3 group when compared to GlcN1 group at different time intervals (after 8, 12 and 16 weeks) even after stoppage of drug treatment. In the meanwhile GlcN1 group showed significant (P<0.001) diminish in the urine Pyr level only during the course of intervention, after 16 weeks there was a rational reduction in the urine Pyr level when compared to base line. The level of Dpyr excretion in urine was significantly (P<0.001) diminishing in GlcN2 and ChoN3 group when compared to GlcN1 group at different time intervals (after 12 and 16 weeks) even after stoppage of drug treatment. Both GlcN2 and ChoN3 received group showed a significant (P<0.001) reduction of Dpyr level in urine at different time intervals (after 8, 12 and even 16 weeks) when compared to base line. Only GlcN1 group showed a moderate reduction of Dpyr level in urine after stoppage (16 weeks) of drug treatment when compared to base line.

There was no difference in the pre and post drug radiographs of the affected knee joint. Table 2 shows radiological scoring of the knee OA for different treatment groups. The earlier reported study showed that the mean joint space width was assessed by some advanced technique like digital image analysis, where as minimum joint-space width i.e, at the narrowest point was measured by visual inspection with a magnifying lens [26]. The patients did not complain about any side effects and ADR during the entire study period. Laboratory investigations such as haemogram, liver and renal biochemical tests were quite normal in all groups of patients before and after the therapy. All the medications were found to be safe and did not lead to any significant alteration in the liver and renal functions. Similarly, the medications were well tolerated by the patients.

Table. 6. Difficulty in performing daily activity scores of GlcN1, GlcN2 and ChoN3 treated OA knee patients at different time intervals.

Time	GlcN1 (n=45)	GlcN2 (n=45)	ChoN3 (n=45)
Base line	555.62 ± 30.12	561.23 ± 42.5	550.7 ± 37.18
After 4 Weeks	510.4 ± 34.4###	468.54 ± 40.25***,###	464.1 ± 41.2***,###
After 8 Weeks	440.3 ± 38.12###	349.52 ± 29.56***,###	345 ± 44.21***,###
After 12 Weeks	380.4 ± 27.19###	219.65 ± 18.65***,###	220.7 ± 34.7***,###
After 16 Weeks	376.8 ± 36.12###	217.84 ± 19.56***,###	218.1 ± 38.7***,###

Values are in mean ± SEM (n=35); ***P<0.001 Vs GlcN1 treated group, ###P<0.001 Vs Base line. (Repeated measure of one-way ANOVA followed by Dunnett's multiple comparison tests)

Table. 7. Levels of Pyridinoline (Pyr) excretion in urine of GlcN1, GlcN2 and ChoN3 treated OA knee patients at different time intervals.

Time	GlcN1 (n=45)	GlcN2(n=45)	ChoN3 (n=45)
Base line	530.81 ± 2.54	529.54 ± 9.53	524.5 ± 1.74
After 8 Weeks	450.12 ± 4.63###	428.7 ± 10.45***,###	430.4 ± 2.41***,###
After 12 Weeks	366.8 ± 7.91###	341.28 ± 2.56***,###	340.1 ± 1.23***,###
After 16 Weeks	354.1 ± 5.17###	337.05 ± 2.05***,###	336.1 ± 4.74***,###

Values are in mean ± SEM (n=35); ***P<0.001 Vs GlcN1 treated group, $^{###}P$<0.001 Vs Base line. Pyr expressed in picomoles per micromole creatinine. (Repeated measure of one-way ANOVA followed by Dunnett's multiple comparison tests).

Table. 8. Levels of deoxypyridinoline (Dpyr) excretion in urine of GlcN1, GlcN2 and ChoN3 treated OA knee patients at different time intervals

Time	GlcN1 (n=45)	GlcN2(n=45)	ChoN3(n=45)
Base line	34.32 ± 3.17	34.56 ± 2.06	32.67 ± 2.85
After 8 Weeks	25.4 ± 1.82##	22.48 ± 1.65###	20.1 ± 2.26**,###
After 12 Weeks	13.67 ± 1.01###	5.75 ± 0.43***,###	6.16 ± 0.49***,###
After 16 Weeks	9.8 ± 0.85###	4.06 ± 0.28***,###	4.81 ± 0.48***,###

Values are in mean ± SEM (n=35); **P<0.01, ***P<0.001 Vs GlcN1 treated group, $^{##}P$<0.01, $^{###}P$<0.001 Vs Base line. Dpyr expressed in picomoles per micromole creatinine. (Repeated measure of one-way ANOVA followed by Dunnett's multiple comparison tests).

DISCUSSION

In olden days, innumerous reported studies suggested that NSAIDs are widely used in the relief of pain in-patients with KOA, despite which produces serious adverse effects associated with their long term use [27]. Today, a cure of KOA is still an enigma. The management of KOA is largely palliative, focusing on the alleviation of symptoms. Current recommendations for the management of KOA include a combination of non-pharmacological interventions (weight loss, physiotherapy, education programs, patient counseling, etc.,) and pharmacological treatments (NSAIDs, etc.,). Jones *et al* reported a post marketing surveillance study of sustained release form of diclofenac on 7438 KOA patients, in which the drug had to be withdrawn in 18 % of the patients due to side effects [6]. In another study involving 336 patients with KOA over six months, Hosie *et al* reported that about 10 % patients withdrew from the study due to adverse effects following diclofenac therapy [4]. KOA is characterized by progressive loss of articular cartilage and bony overgrowth seen mostly in elderly individuals. The initial bland progression of KOA may become clinically relevant as an inflammation brought about by the increasing deposition of cartilaginous debris [28]. For the KOA patient, the most important aspect of the condition is pain and associated impairment of movement [29]. Because cartilage is not innervated, the pain arises from secondary effects, such as synovial inflammation and fluid accumulation leading to joint capsule distention and stretching of the periosteal nerve endings. In this context, there is a need for safe and effective long lasting alternative treatments while the absence of any cure reinforces the importance of prevention. Such, prevention and alternative treatments could come from nutrition. It is now increasing recognized that beyond meeting basic nutritional needs, nutrition supplements may play a beneficial role in some diseases [7]. Glucosamine and chondroitin have been used widely in many studies in KOA all over the world as nutritional supplements aiding cartilage repair and regeneration, found to be uniformly safe in all studies compared to NSAIDs [10,13,30]. A possible reason for the persistent effect of GlcN2 and ChoN3 in KOA patients even four weeks after stopping treatment may be an effect on the underlying pathology in KOA. Kelly GS and Leffier CT *et al* determined that the combination therapy relieves symptoms of knee OA, effectively control pain and reverse progression of the disease [31,32]. Our finding shows that there is statistically significant improvement in the efficacy variables in the patients of OA knee treated with GlcN2 and ChoN3. After 12 weeks of both GlcN2 and ChoN3 therapies decreased the pain in the affected knee joints, decreased swelling and improved the loss of function in terms of increased knee flexion, stairs climbing and walking distance. There was good statistical concurrence of WOMAC scores observed in both GlcN2 and ChoN3 treated OA patients when compared with improvement in symptoms of OA. However, WOMAC scores was the primary outcome measure, and showed changes similar to those we hypothesized in our power calculation. Even though there was a good relief in pain, swelling and performing daily activities significant reduction in WOMAC scores was observed in GSO_4 i.e, GlcN1, which is less comparable with GlcN2 and ChoN3 as per our clinical findings. At the same time its effect was persistent even after four weeks of stoppage of treatment. The main course of action of chondroitin sulfate is to inhibit the breakdown of proteoglycans by helping them retain valuable joint lubricating fluids. It also protects existing cartilage from a premature breakdown, by inhibiting certain enzymes that destroy cartilage and enzymes that prevent the transport of nutrients, and stimulates the production of proteoglycans, glycosamino glycans and collagen, the cartilage matrix molecules that serve as building blocks for healthy new cartilage [14]. Morreale RM reported that chondroitin sulfate seems to produce a slow, but gradually increasing clinical acitivity in OA, and that these benefits last for a long period [33]. A double blind placebo controlled trial explained that chondroitin sulfate reduces pain and improves motility in patients with joint degeneration [34]. This is a valuable finding as most of the currently used drugs in OA from modern medicine provide short lasting symptomatic relief, as also seen by various authors in NSAIDs treated group, where the onset of action was fast but waned rapidly on stoppage of treatment.

In our study the exact effect of a drug on articular cartilage can be exerted by the assessment of bone resorption and disease extent by evaluation of Pyr and Dpyr levels in urine. Mac Donald *et al* found elevated urinary pyridinium crosslinks correleated with OA of the knee and they concluded that these crosslinks markers could serve as useful indicators of disease activity in OA [35].

Robins SP *et al* and Seibel MJ *et al* studied that pyridinium crosslinks such as Pyr and Dpyr found extensively in bone cartilage, which is excreated in the urine in higher amounts when cartilage breaks down, and also stated that this two collagen crosslinks measured in urine provide information about both the pathogenesis of OA as well as the rate of bone turnover so that this pyridinium crosslinks serve as an biomarker to assess joint destruction in OA [20,21, 36-38]. Mac Donald *et al* explined that these crosslinks markers could serve as useful indicators of disease activity in OA [35]. Our study reports of pyridinoline and deoxypyridinoline level with the presence of osteoarthritis and improvement in the quality of life of osteoarthritis patients with the continuation of 12 weeks intervention glucosamine therapy shows consensus with earlier reported studies [20,21,39]. So, this study strongly suggests that the GlcN2 is most effective compound when compared to GlcN1 and ChoN3 for the treatment of OA.

In consequence, it can be affirmed that GlcN2 and ChoN3 can relieve pain, improving functional ability and joint mobility so as to enhance the quality of life of patients with OA of knee. In the mean while, GlcN2 and ChoN3 treated KOA patients showed best response as well as it was cost effective for OA knee patients when compared with GlcN1 alone. Both GlcN2 and ChoN3 showed superior efficacy and long lasting effect was accomplished, when compare to GlcN1 treated KOA patients. In terms of tolerability and safety both of the drugs are good evidenced by the patient compliance and the fact that there was no untoward adverse effect noted during the study.

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Conflict of interest statement

There was no sponsored research involving this study design. Furthermore the authors have full control of all primary data and will agree to allow the journal to review the data if requested.

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