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EVALUATION OF TOLERABILITY AND EFFICACY OF GLUCOSAMINE WITH CHONDROITIN IN THE TREATMENT OF KNEE OSTEOARTHRITIS – A DOUBLE BLIND MULTICENTRE RANDOMIZED PROSPECTIVE COMPARATIVE STUDY

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ABSTRACT: There are few comparative studies conducted with glucosamine [GlcN1 (glucosamine sulfate with potassium chloride), GlcN2 (glucosamine sulfate plus chondroitin sulfate) along with ChoN3 (chondroitin sulfate alone)] in the treatment of knee osteoarthritis. In this study the treated groups were studied for alleviation of pain and joint stiffness with correlation of measurement of urinary pyridinium cross links such as pyridinoline (Pyr) and deoxypyridinoline (Dpyr). Hence, this study was eventually planned to evaluate the efficacy, safety and tolerability of glucosamine with chondroitin sulfate treated groups. Urinary pyridinium crosslinks such as Pyr and 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. The results of this study suggest that GlcN2 and ChoN3 can relieve pain, improving functional ability and joint mobility so as to enhance the quality of life for osteoarthritis patients.

Key words: Osteoarthritis, glucosamine, chondroitin, pyridinoline, pain

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 management of OA is largely palliative, focusing on alleviation of symptoms. Current 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 glycosaminoglycans (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].

On other hand, globally there is greater interest on chondroitin sulfate which consists of repeating chains of molecules called mucopolysaccharides. Chondroitin sulfate is a glycosaminoglycan, which is a major component of the lining of joints and allows attracting and holding the large quantity of water to the cartilage building proteoglycan molecules. Chondroitin sulfate provides nourishment for healthy cartilage and connective tissues, provides supports connective tissue, including ligaments and tendons [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 the substantial improvement when compared with placebo administered clinical trial. GSO_4 was well tolerated, and no adverse effects observed [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 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.

Methods

Study design

The present study was a double blind, multicentric, randomized, prospective, comparative trial. The study protocol was approved by the institutional ethics committee of KM College of Pharmacy, Madurai- 625 107, Tamilnadu, India. Informed written consent was obtained from all 105 patients. The study design, population, intervention and outcome measures based on CONSORT guidelines [19].

Population

105 patients aged between 40 and 70 years of either gender with primary OA of knee, diagnosed according to the criteria of American College of Rheumatology [20] were enrolled from out patient department of Devadoss Orthopaedic Hospital, Vinayagam Nagar, Madurai, Tamilnadu, India and Institute of Sports Medicine, Madurai, Tamilnadu, India. The diagnosis was based on clinical presentation and X-ray verified reduction in interarticular space was evaluated for inclusion in this double blind study by orthopaedician.

Complete clinical evaluation plus hemogram, liver and renal biochemistry were done in all cases. Exclusion criteria included current or recent (less than two weeks) anti-rheumatic therapy, arthrosis secondary to systemic disease, suspected bacterial infection of the joint, existing pregnancy and lactation, known hypersensitivity to active principles or auxillary substances of test drugs. Patients with hemorrhagic disorders, history of peptic ulcer, acid peptic disease, concurrent illness, receiving concomitant drug therapy, history of drug allergy and who had undergone corticosteroid therapy in the last two months were also excluded.

Intervention

105 OA patients randomly allocated (randomization done by SAS system for windows) into three groups of 35 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 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.

Outcome measures

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. Assessment with WOMAC scale at 16 weeks of therapy was done to evaluate the residual effect of treatment. The WOMAC scale is an internationally accepted subjective scale. 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.

Biometric 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).

Limitations of the study

In our study we found that there are some limitations regarding the cost based therapy such as all the patients were not able to afford the actual cost of chondroitin sulfate even we received gift sample from the concern manufacturer and finally the therapeutic effect can be compared with WOMAC scale and measurement of the level of pyridinoline (Pyr) and deoxypyridinoline (Dpyr) in urine of the patients, along with the MRI scan report also will be helpful to elucidate the right correlation between the effect of drug with the level of Pyr and Dpyr. But it is too costly for all patients, this is the reason we did not correlate with MRI scans. If we are able to extending the length of follow up for the next 8 weeks it could be more subjective. The followings are the major reason for drop out of the patients from our study, (i) a few adverse events like vomiting and GIT disturbances was occur during the in take of GlcN1 and GlcN2, (ii) due to high severity of the disease condition three patients were dropped out from the study, (iii) due to other medical or personal reasons like shifting of house, transfer of job, lack of faith to physician and psychological stress eleven patients were dropped out from our study.

RESULTS

In our double blind multicentre clinical study, we compared the efficacy of salt forms of glucosamine containing preparations (GlcN1, GlcN2 and ChoN3) with chondroitin sulfate alone treated group in knee OA patients. Of 105 patients enrolled in the study, 35 patients (13 men; mean age 59 years) received GlcN1, 35 patients (11 men; mean age 57 years) received GlcN2, and 35 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). 84 % 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	105
Number of patients in group I (GlcN1)	**35**
Number of patients in group II (GlcN2)	**35**
Number of patients in group III (ChoN3)	**35**
Number of patients completed the study	**90**
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=35)	GlcN2 (n=35)	ChoN3 (n=35)
Base line	2.40 ± 0.12	2.31 ± 0.14	2.27 ± 0.19
After 12th week	2.24 ± 0.18	1.97 ± 0.17	1.93 ± 0.10

Values are in mean ± SEM (n=35). 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=35)	GlcN2(n=35)	ChoN3 (n=35)
Base line	233.9 ± 7.1	238.4 ± 9.16	235.4 ± 8.01
After 4 Weeks	205.1 ± 5.14$^{\#\#}$	200.89 ± 8.45***$^{\#\#\#}$	203.9 ± 6.45***$^{\#\#}$
After 8 Weeks	140.4 ± 7.4$^{\#\#\#}$	127.45 ± 10.2***$^{\#\#\#}$	130.6 ± 8.1***$^{\#\#\#}$
After 12 Weeks	70.4 ± 6.3$^{\#\#\#}$	60.78 ± 6.5**$^{\#\#\#}$	62.1 ± 11.3**$^{\#\#\#}$
After 16 Weeks	67.8 ± 5.14$^{\#\#\#}$	62.45 ± 5.45***$^{\#\#\#}$	63.8 ± 8.45***$^{\#\#\#}$

Values are in mean ± SEM (n=35); **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=35)	GlcN2 (n=35)	ChoN3 (n=35)
Base line	86.7 ± 4	82.4 ± 7.02	85.38 ± 3.2
After 4 Weeks	63.7 ± 8.2 ##	60.53 ± 5.2*** ###	59.6 ± 4.3*** ###
After 8 Weeks	44.9 ± 6.59 ##	42.56 ± 3.8 ##	41.5 ± 6.78 ##
After 12 Weeks	39.8 ± 3.65 ##	27.5 ± 1.8** ###	27.6 ± 5.3** ###
After 16 Weeks	38.2 ± 8.4 ##	25.5 ±1.92*** ###	26.2 ± 6.3*** ###

Values are in mean ± SEM (n=35); **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*=35)	GlcN2 (*n*=35)	ChoN3 (*n*=35)
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*=35)	GlcN2(*n*=35)	ChoN3 (*n*=35)
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*=35)	GlcN2(*n*=35)	ChoN3(*n*=35)
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

Previously reported studies suggested that NSAIDs are widely used in the relief of pain in-patients with OA, despite which produces serious adverse effects associated with their long term use [27]. Today, a cure of OA is still an enigma. The management of OA is largely palliative, focusing on the alleviation of symptoms. Current recommendations for the management of OA 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 OA 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 OA over six months, Hosie *et al* reported that about 10 % patients withdrew from the study due to adverse effects following diclofenac therapy [4]. OA is characterized by progressive loss of articular cartilage and bony overgrowth seen mostly in elderly individuals. The initial bland progression of OA may become clinically relevant as an inflammation brought about by the increasing deposition of cartilaginous debris [28]. For the OA 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 has been used in many studies in OA 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 OA patients even four weeks after stopping treatment may be an effect on the underlying pathology in OA. 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* described 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.

Thus in conclusion, it can be stated 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 OA 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 better efficacy and long lasting effect was attained, when compare to GlcN1 treated OA 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.

REFERENCES

1. Harrison's. Disorders of immune system, connective tissue and joints. In. Principles of Internal Medicine.The McGraw-Hill companies, USA, 1998:1935-41.
2. Harshmohan. The musculoskeletal system.In.Text Book of Pathology.Lordson publishers, Delhi, 3rd ed, 1998:1004-5.
3. Jordan KM, Arden NK, Doherty M. Eular Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: Report of a task force of the standing committee for international clinical studies including therapeutic trials (ESCISIT). Ann Rheum Dis. 2003; 62: 1145-1155.
4. Hosie J, Distel M, Bluhmki E. Meloxicam in osteoarthritis: a six month, double-blind comparison with diclofenac sodium. Br J Rheumatol. 1996; 35: 39-43.
5. Laine L. Nonsteroidal anti-inflammatory drug gastropathy.Gastrointest Endosc Clin North Am 1996; 50: 390-5.
6. Jones CW. A post-marketing surveliance study of voltarol 75 mg SR in the primary care setting. Br J Clin Pract. 1996; 50: 390-95.
7. German B, Schiffrin EJ, Reniero R, Mollet B. The development of functional foods: lessons from the gut. Trends Biotechnol. 1999; 17: 492-499.
8. Mankin HJ, Johnson ME, Lippiello L. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. III. Distribution and metabolism of amino sugar- containing macromolecules. J Bone Joint Surg. 1981; 63: 131-9.

9. Setnikar I, Giacchetti C, Zanolo G. Pharmacokinetics of glucosamine in the dog and in man. Arzneimttelforschung. 1998; 36: 729-35.
10. Ronca F, Palmieri L, Panicucci P, Ronca G. Anti-inflammtory activity of chondroitin sulfate. Osteoarthritis Cartilage. 1998; 6: 14-21.
11. Bucsi L and Poor G. Efficacy and tolerability of oral chondroitin sulfate as symptomatic slow-acting drug for osteoarthritis (SYSADOA) in the treatment of knee osteoarthritis. Osteoarthritis Cartilage. 1998; 6: 31-36.
12. Reichelt A, Forster RR, Fischer M. Efficacy and safety of intramuscular glucosamine sulphate in osteoarthritis of the knee. Drug Res. 1994; 44: 75.
13. McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. JAMA. 2000; 283: 1469-75.
14. Lakshmi Menon. Chondroitin Sulfate acts like a liquid magnet attracts large amount of water to the proteoglycan molecules. The Medical Scientific Department, Apex Labs Ltd., Chennai, INDIA. 2000; 1-4.
15. Crolle G, D'Este E. Glucosamine sulphate for the management of arthrosis: A controlled clinical investigation. Curr Med Res Opin. 1980; 7: 104-109.
16. D'Ambrosio E, Casa B, Bompani R, Scali G, Scali M. Glucosamine sulphate: A controlled clinical investigation in arthrosis. Pharmatherapeutica. 1981; 2: 504-508.
17. Robins SP, Stewart P, Astbury C, Bird HA. Measurement of the cross linking compound, pyridinoline in urine as as index of collagen degradation in joint disease. Ann Rheum Dis. 1986; 45: 969-73.
18. Seibel MJ, Duncan A, Robins SP. Urinary hydroxy pyridinium crosslinks provide indices of cartilage and bone involvement in arthritic diseases.J Rheumatol. 1989; 16: 964-70.
19. David M, Kenneth FS and Douglas GA. The CONSORT statement: revised recommendations for improving the quality of reports of parallel group randomized trials. BMC Medical Research Methodology. 2001; 1: 2.
20. Hart DJ, Spector TD. The classification and assessment of osteoarthritis. Baillieres Clin Rheumatol. 1995; 9: 407-32.
21. Nicholas B. WOMAC Osteoarthritis Index VA 3.1. User Guide 5. University of Queensland Faculty of Health Sciences: Australia: 2002; 15-16.
22. Elizebeth HF. Degenerative Joint Disease. In: Stuart L, Joseph AB. Turek's Orthopedics- principles and their application. 5th ed. JB Lippincott Company. 155-57.
23. Brandt KD, Fife RS, Braunstein EM, Katz B. Radiographic grading of the severity of knee osteoarthritis: relation of the Kellgren and Lawrence grade to a grade based on joint space narrowing, and correlation with arthroscopic evidence of articular cartilage degeneration. Arthritis Rheum. 1991; 34: 1381-86.
24. Eyre DR, Koob TJ and Vanness KP. Quantitation of hydroxypyridinium crosslinks of collagen by high-performance liquid chromatography. Anal Biochem. 1984: 137: 380-388.
25. Marowska J, Kobylinska M, Lukaszkiewicz J. Pyridinium crosslinks of collagen as a marker of bone resorption rates in children and adolescents: Normal values and clinical application. Bone. 1996; 19: 669-77.
26. Reginster JY, Deroisy R, Royati LC, Lee RL, Lejeune E. Long term effects of glucosamine sulfate on osteoarthritis progression:a randomized, placebo-controlled clinical trial. Lancet. 2001;357:251-56.
27. Abramson SB: The role of NSAIDs in the treatment of osteoarthritis. In Osteoarthritis edited by: Brandt KD, Doherty M, Lohmander LS. OxfordUniversity Press; 2003: 251-258.
28. Niethard FU, Pfeil J. Orthopedie, Hippocrates Vertag, Stuttgart 1989.
29. Maziers B. Gonoarthroses. Rev Prat. 1996; 46: 2193-200 (In French).
30. Smalley WE, Ray WA, Daugherty JR, Griffin MR. Non-Steroidal anti-inflammatory drugs and the incidence of hospitalizations for peptic ulcer disease in elderly persons. Am J Epidemiol. 1995; 141: 539-45.
31. Kelly GS. The role of glucosamine sulfate and chondroitin sulfate in the treatment of degenerative joint disease. Altern Med Rev. 1998; 3: 27-39.
32. Leffier C.T. Glucosamine, chondroitin, and manganese ascorbate for degenerative joint disease of the knee or low back: A randomized, double-blind, placebo-controlled pilot study. Mil Med. 1999; 164: 85.
33. Morreale RM. Comparison of the anti-inflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. J Rheumtol. 1996; 23:1358-91.
34. Oliviero U, Sorrentino GP, De Paola P, *et al*., Effects of the treatment with Matrix on elderly people with chronic articular degeneration. Drugs Exp Clin Res 1991;17:45-51.

35. MacDonald AG, McHenry P, Robins SP, Reid DM. Relationship of urinary pyridinium crosslinks to disease extent and activity in osteoarthritis. Br J Rheumatol. 1994; 33: 16-9.
36. Robins SP, Duncan A, Wilson N and Evans BJ. Standardization of pyridinium crosslinks, pyridinoline and deoxypyridinoline, for use as biochemical markers of collagen degradation. Clin Chem. 1996; 42: 1621-26.
37. Gunja SZ, Boucek RJ. Collagen crosslink components in human urine. Biochem J. 1981; 197: 759-62.
38. Fujimoto D, Suzuki M, Uchiyama A, Miyamoto S, Inoue T. Analysis of pyridinoline, a crosslinking compound of collagen fibers, in human urine. J Biochem. 1983; 94: 1133-6.
39. Thompson PW, Spector TD, James IT, Henderson E, Hart DJ. Urinary collagen crosslinks reflect the radiographic severity of knee osteoarthritis. Br J Rheumatol. 1992; 31: 759-61.