Role of Glucosamine Sulphate, Chrondoitin Sulphate & Vitamin C in enhancing chrondoprotective effects and management of pain in Osteoarthritis

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Dedicated To My Parents
&
Honourable Teachers

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled **"Role of Glucosamine Sulphate, Chrondoitin Sulphate & Vitamin C in enhancing chrondoprotective effects and management of pain in Osteoarthritis"** is an authentic and genuine research work carried out by me under the guidance of Dr. Repon Kumar Saha, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

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Abstract

Osteoarthritis (OA) is a degenerative joint disease involving the cartilage and many of its surrounding tissues. The risk of disability is so great that it is now a costly burden to society and loss of productivity. So, the objectives in managing the patient with OA are to reduce/eliminate pain & stiffness, maintain/improve mobility, optimize function & hence minimize disability. Currently, there is no cure to osteoarthritis. Available treatment options are only aimed at alleviating pain and improving functionality of joints. NSAIDs are recommended as second line treatment (after acetaminophen) for mild OA and as first-line treatment for moderate to severe OA. However, due to the potentially serious adverse reactions of NSAIDs, they are not suitable for long term use. Numerous clinical studies have demonstrated that the targeted administration of selected micronutrients leads to a more effective reduction of OA symptoms, with less adverse events. Consistent with the in vitro and in vivo studies done on glucosamine and chrondoitin sulphate, some clinical trials thus far reported support the demonstrated favorable effects of glucosamine and chondroitin sulfate alone or in combination in relieving OA pain. However, none of a larger number of randomized clinical trials gave such positive results, suggesting an ambiguity of the benefit of these two nutraceuticals in OA. The study has been conducted in 3 groups of 20 patients in order to evaluate and compare improved treatment outcomes combining Paracetamol, Gucosamine hydrochloride, Chrondoitin sulfate & Vitamin C. Superior effects of combination treatment considering paracetamol, glucosamine sulphate, chrondoitin sulphate and vitamin C over glucosamine sulphate, chrondoitin sulphate and paracetamol were seen during the study suggesting the combination of vitamin C with Glucosmine sulphate and chrondoitin sulphate may play a vital role in alleviation of pain in knee OA patients. Since the other treatment options carry a considerable risk of side effects over long term use, this treatment combination maybe a very valuable and safe method of ensuring long term treatment of knee OA with negligible side effects.

List of Abbreviations

OA	Osteoarthritis
USA	United States of America
NHANES	National Health and Nutrition Examination Survey
COPCORD	Community Oriented Program for the Control of Rheumatic Disease
ROAD	Research On Osteoarthritis Against Disability
PRG	Proteoglycan
NSAID	Non-Steroidal Anti-inflammatory Drug
ROS	Reactive Oxygen Species
iNOS	Inducible Nitric Oxide Synthase
PG	Prostaglandin
MMP	Matrix Metalloproteinase
COX	Cyclooxygenase
PPI	Proton-pump inhibitor
GAG	Glycoprotein and Glycosaminoglycan
CS	Chondroitin sulfate
ECM	Extracellular Matrix
TENS	Transcutaneous Electrical Nerve Stimulation
VAS	Visual Analogue Scale
ADL	Activity of Daily Living

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Introduction

Osteoarthritis (OA) is a degenerative joint disease involving the cartilage and many of its surrounding tissues. In addition to damage and loss of articular cartilage, there is remodeling of subarticular bone, osteophyte formation, ligamentous laxity, weakening of periarticular muscles, and, in some cases, synovial inflammation. These changes may occur as a result of an imbalance in the equilibrium between the breakdown and repair of joint tissue. Primary symptoms of OA include joint pain, stiffness, and limitation of movement. Disease progression is usually slow but can ultimately lead to joint failure with pain and disability.[1]

Its aetiology is largely unknown, but is most likely multi-factorial. Osteoarthritis poses a dilemma: it often begins attacking different joint tissues long before middle age, but cannot be diagnosed until it becomes symptomatic decades later, at which point structural alterations are already quite advanced.[2]

1.1 Epidemiology of Osteoarthritis

OA may develop in any joint, but most commonly affects the knee, hip, hand, spine, and foot. In 2005, it was estimated that over 26 million people in the USA had some form of OA.[3, 4] Self-reported physician-diagnosed OA data from the 2004- 2005 Australian National Health Survey is shown in Figure.[4]

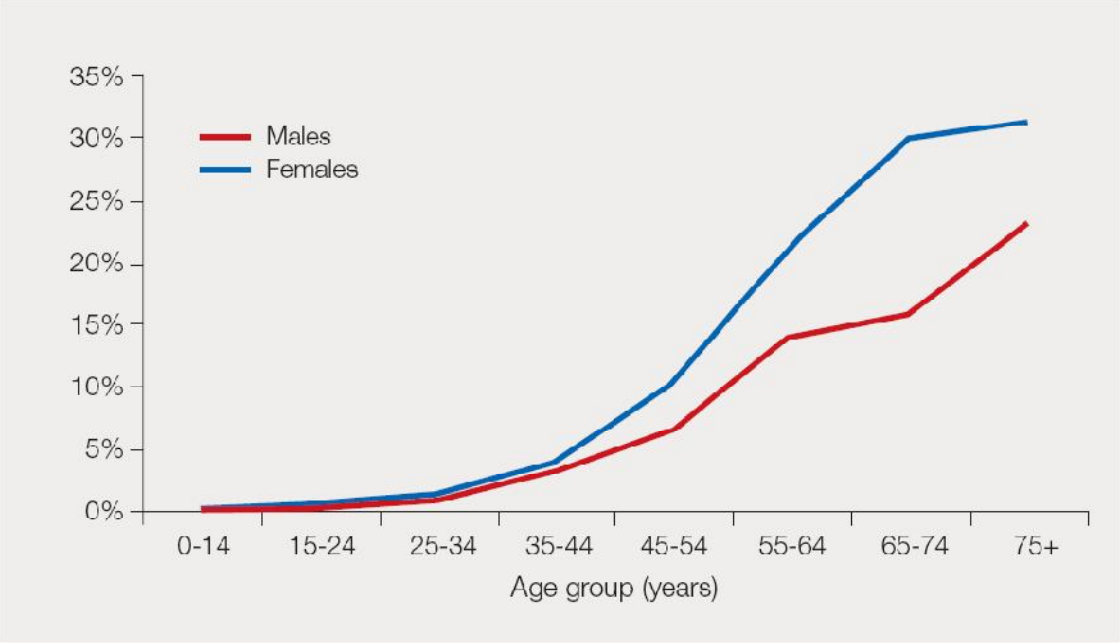

Figure 1.1: Age-specific prevalence of osteoarthritis in Australia in 2004-2005 (Australian Institute of Health and Welfare analysis of the Australian Bureau of Statistics' 2004-2005 National Health Survey).[5]

The incidence of hand, hip, and knee OA increases with age, and women have higher rates than men, especially after the age of 50 years. A levelling off or decline occurs at all joint sites around the age of 80 years. For example, the age- and sex standardized incidence rate from the Fallon Community Health Plan in Massachusetts (USA) was highest for knee OA (240/100 000 person-years), with intermediate rates for hand OA (100/100 000 person-years), and lowest observed rates for hip OA (88/100 000 person-years) [6]

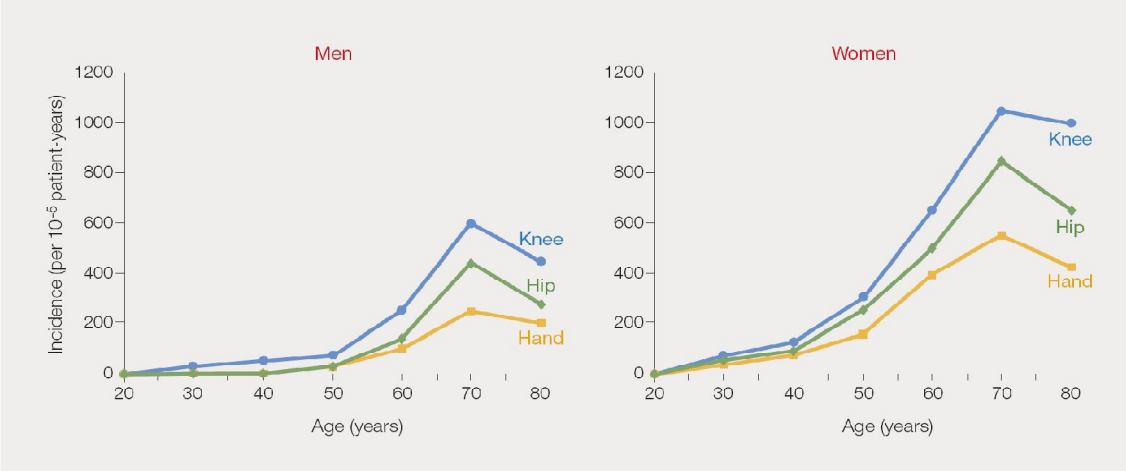

Figure 1.2: Incidence of clinical osteoarthritis of the hand, knee, and hip among participants in the Fallon Health Plan.[6]

The incidence rates found by the Dutch Institute for Public Health (RIVM) in 2000 were similar. For hip OA, the reported prevalence was 0.9 and 1.6 per 1000 per year in men and women, respectively, and for knee OA the corresponding figures were 1.18 and 2.8 per 1000 per year in men and women, respectively.[7]

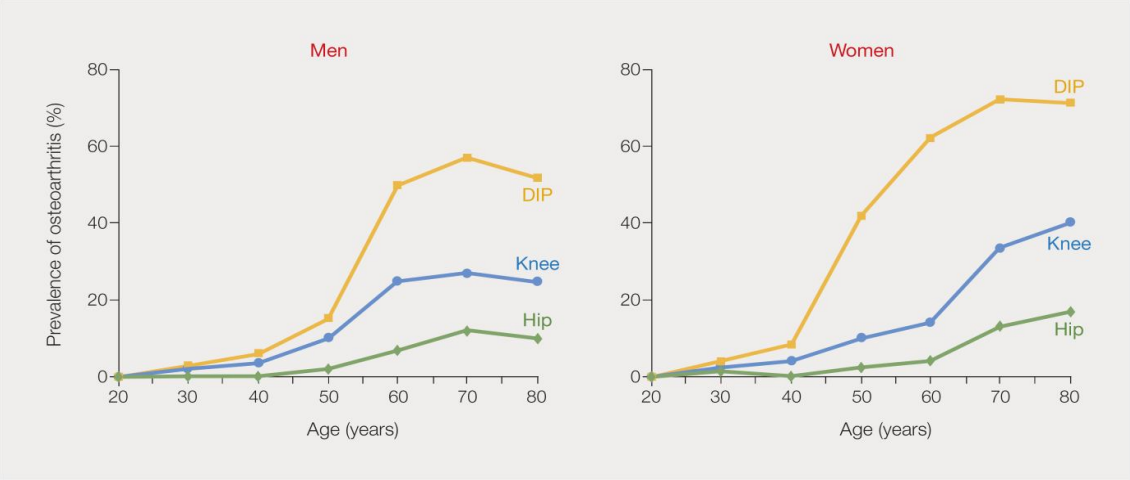

Figure 1.3: Prevalence of clinicalosteoarthritis of the hand, knee, and hip in a Dutch population[7]

1.2 Osteoarthritis of the Knee

Knee involvement occurs less frequently than hand OA, although, similarly, it is more common in women, with female to-male ratios varying between 1.5:1 and 4:1. Prevalence rates for knee OA, based on population studies in the USA, are comparable to those in Europe. These studies report that severe radiographic changes affect 1% of people aged between 25 and 34 years and this figure increases to nearly 50% in those 75 years and above.[8] Few studies have reported secular trends in knee pain; a recent report from the Framingham Study found that the age- and BMI-adjusted prevalence of knee pain and symptomatic knee OA approximately doubled in women and tripled in men over 20 years; no such trend was observed in the prevalence of radiographic knee OA. Similarly, using questionnaire data enquiring about pain in and around the knee, the same researchers found that the age- and BMI-adjusted prevalence of knee pain increased by about 65% in NHANES from1974 to 1994 among non-Hispanic white and Mexican American men and women and among African American women.[9]

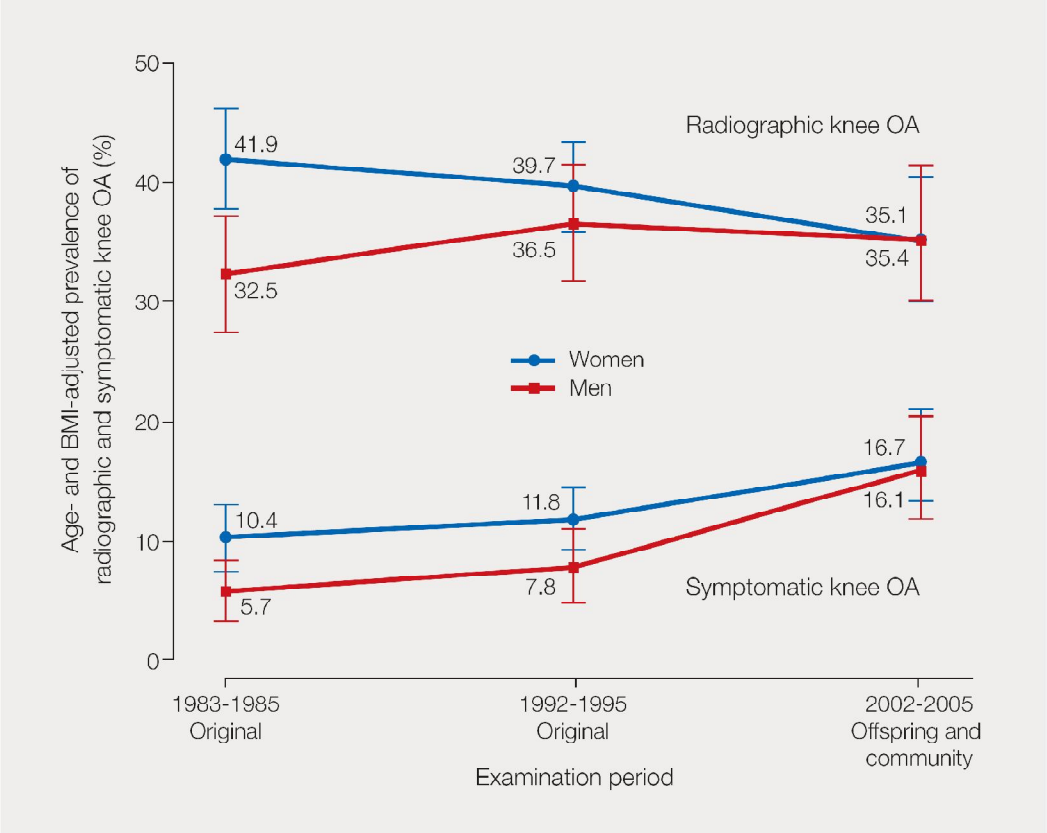

Figure 1.4: Varying prevalence of radiographic and symptomatic knee osteoarthritis over a 20-year period among participants in the Framingham Osteoarthritis Study. [9]

According to data produced by the Dutch Institute for Public Health, the prevalence of knee OA in those aged 55 and above was 15.6% in men and 30.5% in women.[7]

1.3 The epidemiology of osteoarthritis in Asia

Many countries in Asia are ageing rapidly.[10] It has been estimated that the percentage of people aged 65 years and over in Asia will more than double in the next two decades, from 6.8% in 2008 to 16.2% in 2040. In most of the developed world, demographic change was a gradual process following steady socioeconomic growth over several decades. In many Asian countries, the change is being compressed into two or three decades. For example, during the period 2008–2040, it is estimated that Singapore will increase the proportion of people aged 65 and over by 316%, India by 274%, Malaysia by 269%, Bangladesh by 261%, and the Philippines by 256%. In 2008, Japan had the world's oldest population (21.6% aged 65 years and over) and China and India were ranked the top two countries in the absolute number of people aged 65 and over (106 and 60 million, respectively). Apart from ageing, there is much evidence from mostly North American or European cohorts that obesity or heavy occupational physical activity, such as carried out by many people in rural communities within the Asian region, are clear risk factors for symptomatic knee and hip OA.[11]

From the COPCORD studies conducted to-date in the Asian region and providing estimates of knee pain or knee OA, it is evident that the prevalence of either knee pain or knee OA is high, particularly given that the cohorts are quite young, usually 15 years or over, with a mean age mostly between 30 and 39 years.10–24 The COPCORD studies providing age and gender-stratified prevalence estimates generally demonstrate that prevalence increases with age and is higher among women. It is difficult to compare prevalence estimates between the COPCORD studies due to some differences in the screening pain questionnaire terminology and survey methodology, as well as the often incomparable age stratifications reported. The COPCORD studies conducted in India, Bangladesh and Pakistan each collected data from several communities, aiming to detect rural–urban or affluent–poor differences. The two large surveys conducted in India by one group of researchers.[12,13] presented data from these two communities adjusted to the Indian population census of 2001. This adjusted comparison revealed a significantly higher prevalence of knee pain in the rural (13.7%) compared with the urban (6.0%) community.[13] The two surveys conducted in Pakistan demonstrated a higher prevalence of knee pain among the urban affluent compared with the urban poor cohorts within each study.[10]

1.4 RISK FACTORS FOR KNEE OA

Risk factors for knee OA have been studied mostly in Caucasian populations residing in high-income countries and include age, female gender, obesity, a history of knee surgery or significant trauma, or having an occupation requiring heavy lifting, kneeling or squatting.[14] Less epidemiological research in chronic musculoskeletal conditions has been conducted in low and middle-income countries in the Asian region. While it is reasonable to extrapolate some of the risk factor findings from high-income countries to low and middle-income countries, there are also likely to be significant demographic and environmental differences influencing the onset and progression of OA in these regions. Cultural differences of specific importance are the probable lower, though increasing, prevalence of obesity, higher proportion of the population in occupations requiring heavy physical labour, squatting, kneeling and climbing, less access to healthcare and social welfare services, variation between cultures in the way pain is perceived and linguistic variation in the way pain is defined and classified.[15,16,17] Recognition of probable demographic and environmental differences has driven the recent development of a questionnaire identifying risk factor profiles specific for the Asia-Pacific region. The proposed questionnaire includes unique items such as exposures to: religious activities (praying and other sitting religious worships squatting; duration of heavy physical activity; type of toilet; and sitting on the floor (criss-cross, lotus or applesauce, for home activities).[17]

1.4.1 Age, Gender, Obesity

Several large population-based cohort studies conducted in China, Japan, Korea and Pakistan have confirmed an increased risk of symptomatic knee OA associated with older age, female gender and obesity.[18,19,20,21,22,23]

1.4.2 Squatting or Kneeling

An analysis of the ROAD study, conducted in Japan, demonstrated that occupations involving squatting or kneeling more than 2 h per day were associated with an approximately two-fold significantly increased risk of moderate to severe radiographic knee OA (Kellgren Lawrence grade ‡ 3). From the cohort study conducted among people aged 60 years or older in Beijing,25 prolonged squatting at 25 years of age (> 1 h per day) was a common activity and was found to be a strong risk factor for OA of the tibio-femoral joint of the knee. In this analysis, people who reported squatting more than 3 h per day, compared to those who reported squatting < 30 min a day, had twice the likelihood of tibio-femoral OA. The study concluded that prolonged squatting accounted

for a substantial proportion of the difference in knee OA prevalence between Chinese subjects in Beijing and White subjects participating in the Framingham OA study.[24]

1.4.3 Stair-Climbing

A case–control study of hospitalized hip or knee OA patients conducted in Hong Kong demonstrated that a history of joint injury, frequent stair-climbing (15 or more flights per day) or frequent lifting of heavy weights (10 kg or more) were all associated with knee OA.[25] Somewhat in contrast, another study in China[19] reported that people aged 35–64 years living in multi-storey buildings without elevators had a significantly higher prevalence of knee pain compared with those living in single-storey homes (10.1% and 6.5%, respectively); however no correlation between knee OA and climbing stairs could be demonstrated. Interestingly, data from the ROAD study suggest that living in a rural mountainous area doubled the likelihood of symptomatic knee OA (confirmed by radiographs) compared with living in a seaside or urban region.[22]

1.5 Economic burden of osteoarthritis

In 2007, OA increased the probability of missed workdays by 14% in women and by 12% in men. The magnitude of the effect of OA on workdays lost was larger than that for other common conditions such as anxiety disorder, asthma, or diabetes. OA increases annual per capita absenteeism costs by $469 for women and $520 for men – approximately three lost workdays. Extrapolated to the entire country (USA), OA increases absenteeism costs by $10.3 billion per year -- $5.5 billion for women and $4.8 billion for men.[26]

1.6 Pathophysiology of osteoarthritis

Articular cartilage functions as a wear-resistant, smooth, nearly frictionless, load-bearing surface. The composition and physiochemical properties of articular cartilage, the fundamental organization of the collagen network, and the molecular organization of collagen and proteoglycans all have profound effects on the intrinsic mechanical properties of the extracellular matrix.[27] Cartilage is composed of a complex extracellular matrix of collagen and elastic fibers within a hydrated gel of glycosaminoglycans and proteoglycans. This extracellular matrix, which makes up 98% of the articular cartilage volume, is synthesized by the chondrocytes which comprise the other 2% of the cartilage tissue. It is well known that chondrocytes can synthesize the extracellular matrix such as proteoglycans, collagen, fibronectin, integrins, and other adhesive proteins which are needed to maintain the high tensile strength and low compressibility under load of the articular cartilage.[28,29] Type II collagen is the predominant collagen type in the extracellular matrix with proteoglycan (PRG) macromolecules dispersed throughout.

They contain highly negatively charged carboxyl and sulfate groups (keratin and chondroitin sulfate) on the glycosaminoglycans, giving them a high affinity for water. The nature of the high density of negative charges imparts the physical properties to PRGs. Because of their attraction and binding of water, PRGs are viscous, making them ideal for lubricating fluid in joints. The charges repel each other, which gives them an open structure and is space-filling. These biochemical traits contribute to the mechanical properties of PRGs in articular cartilage, such as absorption and distribution of compressive weight, protecting structures in the joints from mechanical damage. The normal synthesis and breakdown of the PRGs and their component molecules, including glycosaminoglycans, is mediated by the indigenous chondrocytes. Glycosaminoglycans turn over several times as rapidly as the fibrillar collagen. If any part of this complex system is disrupted, the normal properties of articular cartilage are jeopardized, leading to joint degeneration. It is the extracellular matrix of articular cartilage that is the primary target of osteoarthritic cartilage degeneration.[30,31]

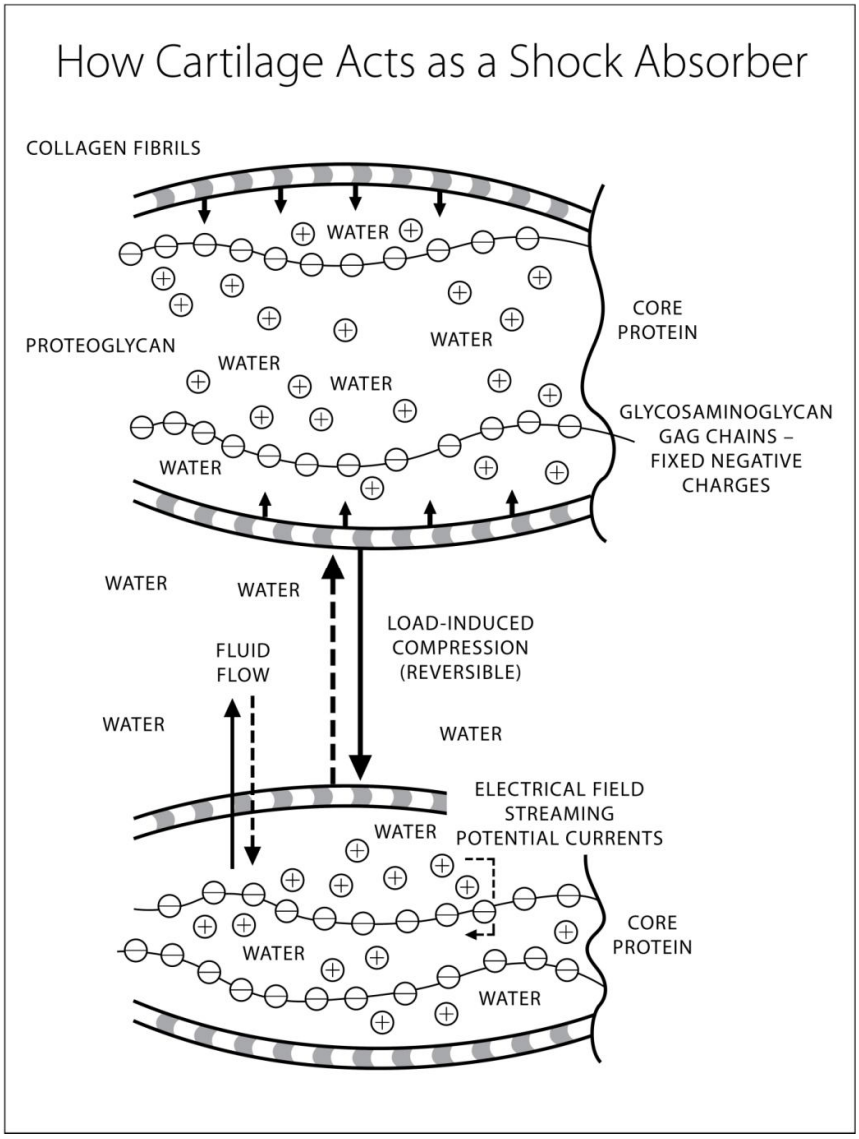

Figure 1.5: The proteoglycan structure of articular cartilage. The high content of water in proteoglycans help the cartilage act as a shock absorber.

One of the earliest features of the development of osteoarthritis is degeneration of the articulating surfaces of the joint. This is characterized by fibrillation of the articular cartilage, in which the mesh of collagen fibers is disrupted. Degeneration of type II collagen is seen, as well as a decrease in the extracellular matrix.[31] Loss of proteoglycan from the matrix is characteristic. The loss of aggrecan, the predominant PRG in articular cartilage imposes an increasing load on the collagen fibrils, causing further breakdown. Early in the course of OA, the tissue mounts an attempt at repair. Chondrocytes proliferate and there is an increase in matrix synthesis.[32] However, if this repair process is disrupted for any reason including the use of NSAIDs, degradative enzymes overwhelm the synthetic capability and the repair fails. Particular compositional,

molecular, and structural changes will continue to occur within the articular cartilage including decreased proteoglycan and increased water content, collagen fibril network disorganization, and proteoglycan separation, as long as the inciting issue (NSAID use) continues. These changes alter the intrinsic mechanical properties of articular cartilage and produce swelling.[33] The articular cartilage, having lost some of its compressive ability under load, further degenerates. As the surface fibrillation progresses, the articular defects penetrate deeper into the cartilage until the cartilage is lost. The increased pressure on the subchondral bone causes it to thicken. Often bone cysts form deep to the eburnated areas. Eventually, bony nodules or osteophytes form at the periphery of the cartilage surface. All of these changes account not only for the pathology found on radiographs or histologically (findings under the microscope), but also for the joint pain, tenderness, loss of motion and stiffness of OA.[34] It is the relief of some of these clinical manifestations that accounts for the widespread use of NSAIDs not only in the United States, but around the world.

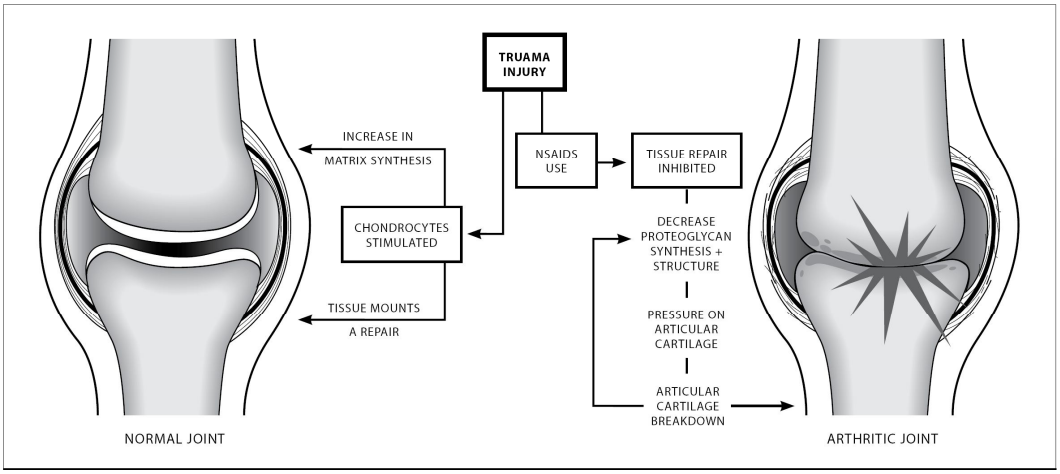

Figure 1.6: The pathogenesis of osteoarthritis that can be accelerated by NSAIDs. NSAID use inhibits the body's repair processes, leading to decreased proteoglycan and extracellular matrix content and function, which ultimately leads to articular cartilage breakdown.

1.7 Inflammation and Reactive Oxygen Species: New Metabolic Approaches to Osteoarthritis

While OA is not synonymous with inflammatory arthropathy, new results indicate that inflammation is not only a secondary event, it is involved in the development of OA from the very beginning.[79, 80,81] Many inflammatory mediators are expressed in the cartilage and synovial tissue in early OA stages. The findings of Benito[81] indicate that inflammatory mediators and nuclear transcription factors involved in the inflammatory

cascade are significantly higher in early-stage OA patients, when compared to late-stage OA. Additionally, reactive oxygen species (ROS) increase during OA[82]. The various inflammatory and oxidative processes in OA are summarized in figure below:

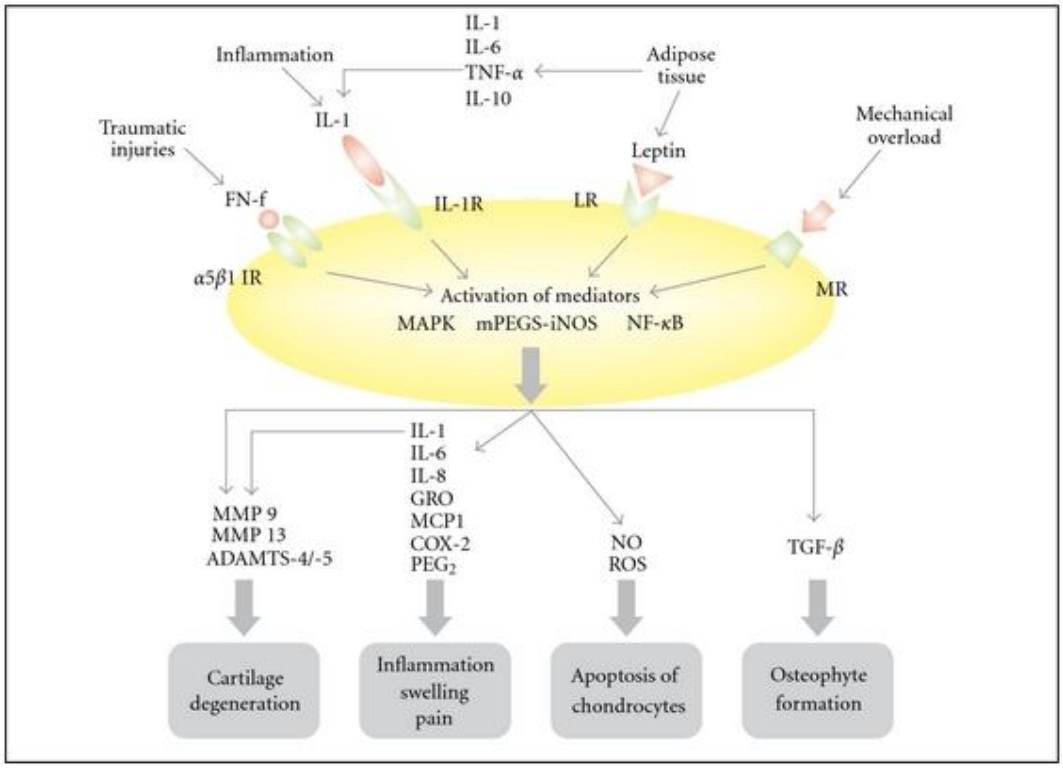

Figure 1.7: Role of inflammatory mediators and oxidative processes in osteoarthritis

Many studies have identified overweight (BMI 25–29.9 kg/m^2) and obesity (BMI >29.9 kg/m^2) as major OA risk factors. Hart and Spector [83] showed that a BMI increase of 2 units will increase the risk of knee OA manifestation by 36%. This is not only due to the additional weight and mechanical stress on the joints, as nonweight-bearing joints—such as the hands—are significantly more affected in patients with high BMI[81], due to metabolic reactions. These include increased inflammation, induced by leptin and other adipocytokines, and dietary lipids or lipid peroxidation, which can lead to cartilage destruction. Therefore, OA is not induced by biomechanical factors and age alone, and several metabolic factors are also involved [84,85]

Leptin is overexpressed in obese patients and is present in the synovial fluid, as well as articular chondrocytes.[86] Excessive and pathological concentrations of leptin, however, like those found in obese patients, have an opposite effect on chondrocytes, cartilage,

and bone, leading to osteophyte formation and cartilage degeneration. Osteophytes in the joints usually limit joint movement and thus provoke pain.[87]

In vitro experiments have elucidated several mechanisms by which excessive amounts of adipokines lead to the destruction of articular joints. In cartilage derived from human OA patients, leptin enhances the synthesis of several proinflammatory mediators, such as NO, PGE_2, IL-6, and IL-8, via inducible nitric oxide synthase (iNOS) pathways. By inhibiting the iNOS activity, NO synthesis was nearly completely blocked. This reduction of NO reduces the production of PGE_2, IL-6, and IL-8 [109]. Furthermore, membrane bound prostaglandin E synthase 1 (mPGES-1) and COX-2 enzyme are overexpressed in the cartilage of such patients. COX-2 further increases the production of prostaglandins. This over expression can be induced by IL-1 and TNF-alpha, factors released by adipose tissue. mPGES-1 mediates the production of PGE_2.[88] PGE_2 overproduction enhances NO-induced cell death of OA chondrocytes. When IL-1 acts together with leptin, they can activate nitric oxide synthase type II, which increases NO production in chondrocytes. Elevated NO levels lead to various catabolic processes in the cartilage, such as the loss of chondrocyte phenotype, thereby reducing production of ECM, and to chondrocyte apoptosis, and ECM degradation. [89,90,91]

Leptin induces the synthesis of matrix metalloproteinases (MMP), especially MMP9 and MMP13, via IL-1 and TNF-alpha. MMPs are a large family of enzymes that degrade different components of collagen and proteoglycans. These experiments clearly show that obesity, mediated by leptin, exerts a proinflammatory and catabolic effect on cartilage, leading to apoptosis of chondrocytes and the degradation of the extracellular matrix.[92]

Thus, overweight and obesity play an important role in the genesis of knee and hip joint OA not only as a result of mechanical overload but also by the complex combined action of genetic, metabolic, neuroendocrine, and biomechanical factors and represent a significant modifiable risk factor. [93]

1.8 Current Treatment Options of Osteoarthritis

Conventional pharmacological approaches to symptom management in OA involve the following:

- Paracetamol
- nonsteroidal anti-inflammatory drugs,
- selective cycloxygenase-2 inhibitors,
- and intra-articular injection of hyaluronan or corticosteroids.

However, there are accumulating data showing that any of these pharmaceutical drugs frequently produce insufficient benefit, with an associated risk of untoward side effects.[35,36,37]

It is therefore no wonder that patients with OA have embraced complementary and alternative approaches such as combination of glucosamine and chrondoitin sulphate to management of OA symptoms, particularly pain.[38,39]

1.8.1 The widespread use of NSAIDS and their adverse effects

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs in the world for the treatment of osteoarthritis (OA) symptoms,[40] and are taken by 20-30% of elderly people (defined as people over the age of 64 years) in developed countries.[41] The worldwide pain management prescription drug market was approximately $24 billion in 2002 and passed $30 billion by 2006. Celebrex (celecoxib) was nearly $4 billion in sales in 2002.[42]

Each year, over 70 million prescriptions for NSAIDs are dispensed in the United States, 20 million in Great Britain and 10 million in Canada.[43,44,45] These numbers do not include the 30 billion over-the-counter tablets sold each year in the United States alone.[46,47]

Treatment guidelines in the United States, Great Britain, and Canada recommend NSAIDs as second line treatment (after acetaminophen) for mild OA and as first-line treatment for moderate to severe OA.[48,49]

Based on the evidence of potentially serious adverse reactions to NSAIDs, the committee has advised against the long-term use of NSAIDs to treat OA. One of the most serious adverse reactions to NSAIDs, that is little appreciated, is that as a class of compounds they cause the breakdown of articular cartilage, thereby accelerating OA, the very disease for which they are most commonly prescribed.

In the normal joint, there is a balance between the continuous process of cartilage matrix degradation and repair. In OA, disruption of the homeostatic state occurs and the catabolic (breakdown) processes of chondrocytes are increased. The principal cytokines linked to the catabolism of cartilage and to the OA process are interleukin (IL)-1, tumor necrosis factor (TNF)-a, and IL-6. IL-1 is the prototypic inducer of catabolic responses in chondrocytes. This substance causes the increased secretion of proteinases (whicbreakdown cartilage matrix) including collagenase, the suppression of proteoglycan synthesis leading to the suppression of matrix synthesis, and ultimately the reduction of the number of chondrocytes. IL-1 is a potent inducer of prostaglandin (PG) synthesis by inducing PGE2 synthesis in human chondrocytes. The rate-limiting step for the synthesis of PGE2 and other prostaglandins is the conversion of arachidonic acid to prostaglandin endoperoxide by cyclooxygenase (COX), which exists in two isoforms, COX-1 and COX-2. All NSAIDs inhibit both COX 1 and 2 enzymes but most of the NSAIDs that have been developed in recent years show greater activity of COX 2 in order to decrease gastrointestinal side effects.

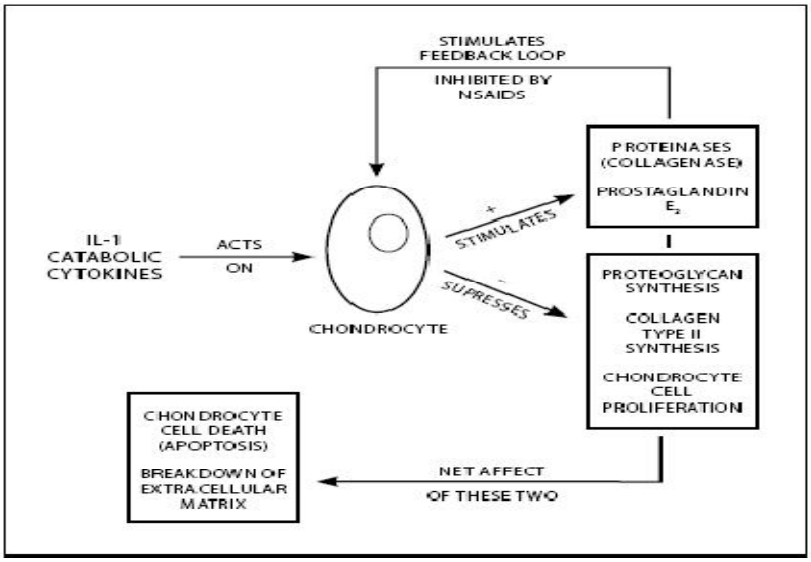

Figure 1.8: The catabolic physiology leading to articular cartilage breakdown. Interleukin-1 is one of the principle cytokines that initiates a cascade that leads to chondrocyte cell death and extracellular matrix breakdown. NSAIDs inhibit prostaglandins, such as PGE2, from stimulating chondrocyte DNA matrix synthesis thereby contributing to articular cartilage degeneration.

PGs act (among other things) as messenger molecules in the process of inflammation. It was hoped that the use of NSAIDs would decrease the catabolic program in OA, thereby having a disease-modifying effect. Research, unfortunately is showing PGs, like PGE2, stimulate chondrocyte proliferation and subsequent synthesis of cellular matrix

components. The net result of their blockade and other NSAID effects is the acceleration of articular cartilage degeneration.[51]

While the prescribing patterns for specific NSAIDs have changed over the years, as drugs like ibuprofen and naproxen became available over-thecounter, an NSAID is still the number one medication prescribed by physicians for osteoarthritis. For instance, 80% of rheumatologists noted they frequently prescribe NSAIDs for symptomatic hip and knee osteoarthritis, while for the same group of clients, 65% of primary care physicians use an NSAID. Even when physicians were educated on guidelines based on the European League Against Rheumatism, American College of Rheumatology, and The Arthritis Society guidelines for OA treatment, limiting NSAID use, NSAIDs were still prescribed over half the time for patients with knee OA.[52] NSAIDS only provide short term relief from the OA pain. On a long term the above adverse effects clearly shows that NSAIDS should not be considered.

Usually in order to prevent epigastric distress, NSAIDS and PPIs are prescribed together. As PPIs have become more widely used, concerns have emerged regarding their potential for adverse effects and long-term harm. One adverse effect that has received increasing attention is osteoporotic fractures. Several observational studies have shown an association between long-term PPI use and fractures of both the hip and vertebrae. This increased risk is thought to be due to achlorhydria, leading to mal-absorption and deficiencies of calcium and vitamin B_{12} and subsequent bone loss.[53]

1.9 Treatment option considering chrondoprotectives and antioxidants

The treatment with chondroprotectives, such as glucosamine sulfate, chondroitin sulfate, hyaluronic acid, collagen hydrolysate, or nutrients, such as antioxidants and omega-3 fatty acids is a promising therapeutic approach. Numerous clinical studies have demonstrated that the targeted administration of selected micronutrients leads to a more effective reduction of OA symptoms, with less adverse events. Their chondroprotective action can be explained by a dual mechanism: (1) as basic components of cartilage and synovial fluid, they stimulate the anabolic process of the cartilage metabolism; (2) their anti-inflammatory action can delay many inflammation-induced catabolic processes in the cartilage. These two mechanisms are able to slow the progression of cartilage destruction and may help to regenerate the joint structure, leading to reduced pain and increased mobility of the affected joint. [54]

Glucosamine, hyaluronic acid, and chondroitin sulfate are important basic natural components of cartilage and synovial fluid. They are naturally formed by the body, but can also be provided in the diet. Supplementation of such basic components may be

beneficial, especially when there is a disturbed balance between catabolic and anabolic processes, such as in osteoarthritis. During OA progression, the chondrocytes are no longer able to fully compensate for the loss of collagen type II fibers and proteoglycans, even at increased synthesis rates.[55] It has been shown in many in vitro and in vivo trials and in numerous clinical studies that these chrondoprotectives can modify, stabilize, retard, or even reverse the pathology of OA.

1.9.1 Glucosamine Salts

Glucosamine or 2-amino-2-deoxy-D-glucose ($C_6H_{13}NO_5$) is an amino monosaccharide. It is synthesized from glucose in almost every human tissue and is most abundant in connective tissue and cartilage. It is an important precursor of the glycoprotein and glycosaminoglycan (GAG) synthesis. Within cartilage, it is most important for the formation of hyaluronic acid, chondroitin sulfate as well as keratan sulfate, which are—aside from the collagen fibers—the most important components of the extracellular matrix of the articular cartilage and the synovial fluid. Glucosamine production is the rate-limiting step in GAG synthesis, and glucosamine supplementation may overcome this bottleneck.[54]

In vitro studies on isolated chondrocytes, or cartilage explants from healthy or OA patients, provide much evidence for the proposed mechanisms regarding how glucosamine supports joint health. It has been shown that glucosamine enhances the production of cartilage matrix components in chondrocyte culture, such as aggrecan and collagen type II.[56,57] Glucosamine increases hyaluronic acid production in synovium explants. Further experiments have shown that glucosamine prevents collagen degeneration in chondrocytes by inhibiting lipoxidation reactions and protein oxidation.[58] MMPs (matrix metalloproteinases) and aggrecanases are the predominant cleavage enzymes in the cartilage. These enzymes are responsible for cleavage preferentially in the interglobular domain of the aggrecan molecule, which leads to loss of aggrecan function. Glucosamine is able to inhibit the MMP synthesis, and further proteoglycan degeneration is therefore prevented. Glucosamine also inhibits aggrecanase by suppression of glycosylphosphatidylinositol-linked proteins.[59,60]

1.9.2 Chondroitin Sulfate

Chondroitin sulfate (CS) is one of the natural glycosaminglycans (GAG) composed of the alternating sugars D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc). It is an important component of the extracellular matrix (ECM). CS is the most frequent GAG in the aggrecan molecule of the cartilage. Due to the negative charge of CS, it is responsible for the water retention of the cartilage, which is important for pressure

resistance. The treatments with these chondoprotectives, other than analgesics and NSAIDs, become noticeable after 2 to 3 weeks of regular intake and has a prolonged effect that remains for up to several months.[61]

1.9.3 Modulation of Inflammation Processes and Oxidative Stress Involved in Osteoarthritis by nutrients

The complex relationship between obesity and OA shows that overweight certainly represents the most significant modifiable risk factor for avoiding knee or hip joint OA. Weight reduction and weight stabilization on the basis of a balanced diet with low energy density is crucial in manifest OA. But also the metabolic processes can be influenced by a dietary therapy which mainly includes chondroprotectives, such as glucosamine and chondroitin sulfate or omega-3 fatty acids. [62]

An alternative treatment to the common NSAID therapy for OA is the use of so-called nutraceuticals, such as glucosamine, chondroitin sulfate, hyaluronic acid, hydrolyzed collagen, and omega-3 fatty acids and various vitamins and minerals. In addition to cartilage metabolism stimulation and thereby cartilage regeneration, many of them possess mechanisms which modulate the inflammatory events and oxidative processes involved in OA. As they are components of natural foods, they have far fewer adverse effects in long-term use than NSAIDs or COX-2 inhibitors, as shown in many clinical trials. They interfere with the inflammatory scenario, illustrated in figure 1.7, at various points.

The glucosamine and chondroitin sulfate combination suppresses IL-1-induced gene expression of iNOS, COX-2, mPGEs, and NF-κB in cartilage explants. This leads to reduced production of NO and PGE_2, two mediators responsible for the cell death of chondrocytes and inflammatory reactions[63]. There are several ways by which glucosamine or chondroitin sulfate reduce synthesis of the COX-2 enzyme. Inhibition of the IL-1 beta induced NF-κB pathway by glucosamine sulfate results in reduced synthesis of the COX-2 enzyme.[64] CS alone diminishes the nuclear translocation of NF-κB, which reduces the formation of proinflammatory cytokines IL-1beta and TNF-alpha and proinflammatory enzymes such as cyclooxygenase 2 (COX-2) and nitric oxide synthase-2 (NOS-2) [65]

The anti-inflammatory capability of CS was also tested in a rabbit atherosclerosis model. In that model, CS reduced the proinflammatory molecules C-reactive protein and IL-6 in serum, as well as the expression of MCP-1 and COX-2 in the peripheral blood mononuclear cells. It also influenced NF-κB that is responsible for the induction of inflammatory processes.[66]

In addition to their anti-inflammatory action, glucosamine and chondroitin sulfate exhibit an antioxidant action which leads to a significant reduction in iNOS expression and activity.

This is one explanation why glucosamine and chondroitin reduce the otherwise NO-induced cell death of chondrocytes. In comparison to glucosamine and CS, hyaluronic acid exerted a very minor anti-inflammatory and antiapoptotic effect, while it significantly reduced NO levels. [67,68]

1.9.4 Ascorbic Acid (Vitamin C)

Ascorbic acid stimulates collagen synthesis and modestly stimulates synthesis of aggrecan (a proteoglycan present in articular cartilage). Sulfated proteoglycan biosynthesis is significantly increased in the presence of ascorbic acid.[69] In human plasma, ascorbate is the only antioxidant that can completely protect lipids from detectable peroxidative damage induced by aqueous peroxyl radicals. Ascorbate appears to trap virtually all peroxyl radicals in the aqueous phase before they diffuse into the plasma lipids. Ascorbate is a highly effective antioxidant, as it not only completely protects lipids from detectable peroxidative damage, but also spares alpha-tocopherol, urate, and bilirubin.[70]

1.9.4.1 Evidence from Animal Studies

In guinea pigs, which, like humans, cannot make vitamin C, supplementation with vitamin C had a protective effect on experimentally induced cartilage degeneration of the knee. Schwartz et al investigated the effect of variation in dietary ascorbic acid on surgically induced OA in the stifle joints of guinea pigs. The animals maintained on the high vitamin C level consistently showed less severe joint damage than animals on the lower level. In a later experiment, Meacock et al studied the appearance and progression of surgically induced OA in the cartilage of the hind knees of guinea pigs. The animals were maintained on either a standard diet or a diet containing extra ascorbic acid in drinking water. It was reported that the extra ascorbic acid had a slight chondroprotective effect on the development of spontaneous lesions.[71,72]

1.9.4.2 Evidence from Human Studies

In the Framingham Osteoarthritis Cohort Study, a moderate intake of vitamin C (120-200 mg/day) resulted in a three-fold lower risk of OA progression. The association was strong and highly significant, and was consistent between sexes, among non-supplement users, and among individuals with different severities of OA. The higher vitamin C intake also

reduced the likelihood of development of knee pain. Vitamin C had no significant effect on the incidence of OA.[73]

1.10 Rationale of the study:

As discussed above, osteoarthritis is a fairly common cause of disability in older people. The risk of disability is so great that it is now a costly burden to society and loss of productivity. So, the objectives in managing the patient with OA are to reduce/eliminate pain & stiffness, maintain/improve mobility, optimize function & hence minimize disability. As per the previous discussion one of the most prescribed medications in OA are NSAIDS which have significant side effects on long term use.

Glucosamine hydrochloride and Chrondoitin sulfate has been used in OA for a long time now. Consistent with the in vitro and in vivo studies done on glucosamine and chrondoitin sulphate, some clinical trials thus far reported support the demonstrated favorable effects of glucosamine and chondroitin sulfate alone or in combination in relieving OA pain.[74,75,76,77,95] However, none of a larger number of randomized clinical trials gave such positive results, suggesting an ambiguity of the benefit of these two nutraceuticals in OA.[78]

Therefore, the rationale for this study would be to see improved treatment outcomes combining Gucosamine hydrochloride, Chrondoitin sulfate & Vitamin C and also see the impact of this treatment in reduction of analgesic use compared to placebo group.

Methodology

2.1 Study design

This is a prospective, randomized single blinded clinical trial to study and differentiate the efficacy of the combination therapy of glucosamine sulfate, chrondoitin sulfate and vitamin C in knee osteoarthritis compared to glucosamine sulfate, chrondoitin sulfate and paracetamol.

The study was carried out from June 2013 to December 2013 and was conducted in the Physical Medicine Department of Dhaka Medical College Hospital. Dhaka Medical College Hospital is a tertiary care hospital in Bangladesh with complete PMR unit with almost all modalities of physical therapy including TENS and all necessary pathological and imaging facilities.

Patients coming to Dhaka Medical College Hospital for treatment of knee osteoarthritis were made to undergo a full clinical examination performed by the physician on duty in order to evaluate the symptoms and possibility of including the patients to the study.

Patients were selected according to the below mentioned inclusion and exclusion criteria:

2.1.1 Inclusion criteria:

Based on informed consent , patients were selected based on the following criteria: Pain in anyone of knee joint, Duration of pain >3 months, Age between 40-70 years, Morning stiffness < 30 minutes, Crepitus on active movement, Bony tenderness, ESR < 40 mm in 1st hour, Radiological evidence of OA knee like marginal osteophytes, subchondral sclerosis, cyst, joint space narrowing and osteochondral loose bodies.

2.1.2 Exclusion criteria

Exclusion criteria were selected based on factors that may hamper the evaluation in this study and included: History of trauma / fall/ sports injury of knee joint, Genu varus / genu valgus deformity, History of knee surgery, Inflammatory arthritis like RA, Spondyloarthropathy, Infectious disease like Tuberculosis, Crystal associated artrhropathy like Gout, Pseudogout, Skin infection over knee joint, Uncontrolled Diabetes Mellitus, surgical treatment of knee joint(s) undergone or its necessity; routine use of health food or medicine containing hyaluronic acid, glucosamine and/or chondroitin sulfate and expected to be continued during the study period; treatment with bisphosphonates, hormones or other medicines that may affect the serum or urine concentrations of biomarkers of bone or cartilage metabolism; intra-articular hyaluronic acid within 2 weeks or corticosteroids within 3

months before inclusion; need to undergo such topical or systemic pharmacological treatments during the study period; occasional taking of hard exercise; a history of osseous or articular diseases other than OA within the past 3 months; treatment with warfarin, undergoing or needed to undergo during the study period; bronchial asthma or potential for developing allergy to the test supplement; pregnant women; nursing mothers or women of childbearing potential.

2.2 Treatment and subject assignment

The selected patients were then randomly allotted to one of the below 3 treatment groups. Group A patients were given Ace 500 mg Tablets (Paracetamol), Contilex Tablets (Glucosamine sulfate 250 mg and chondoitin sulfate 200 mg) 2 tablets, thrice daily and Ceevit Tablet (Ascorbic acid and Sodium ascorbate equivalent to 250 mg Vitamin C) once daily. Group B patients were given Ace 500 mg Tablets (Paracetamol), Contilex Tablets (Glucosamine sulfate 250 mg and chondoitin sulfate 200 mg) 2 tablets, thrice daily. Group C patients were given Ace 500 mg Tablets (Paracetamol).

Patients of all groups were instructed to refrain from taking Ace Tablets as much as possible and to take it only if necessary due to knee pain and not to exceed 3 tablets per day. All patients were instructed not to take any other analgesics during the trial period and were given an outline on the side effects/adverse effects of long term use of Pain killers such as Paracetamol as well as NSAIDS.

ADL instructions (Activity of Daily living) were given to all patients on the baseline date (week 0). Patients were instructed to refrain from putting excess stress on the knee joints. (i.e. not to kneel or sit in squatting position or lift heavy objects).[14] Patients found to be overweight or obese were instructed to lose weight.[98]

The patients were also trained to do various types of muscle strengthening/isometric exercises with the goal of improving muscular balance which helps to reduce the load on the joint., as a non-pharmacologic mode of treatment by on duty physiotherapists in the hospital gymnasium.[97] The patients were advised to do the exercises at least 30 minutes every day. Patients were also advised to apply heat therapy to their knees.

All medicines were donated by Square Pharmaceuticals Ltd., Bangladesh for research purpose and were provided from Dhaka Medical College Hospital to the patients on a 2 weekly basis in order to ensure that the patients visited the facility every 2 weeks.

2.3 Efficacy assessment

The selected patients were then assessed for the following on the baseline date and every subsequent 2 weeks for a period of 8 weeks based on the JOA criteria[97]:

- Pain at rest (with the help of Visual analogue scale)
- Pain while walking (with the help of Visual analogue scale)
- Pain while climbing up or down stairs (with the help of Visual analogue scale)

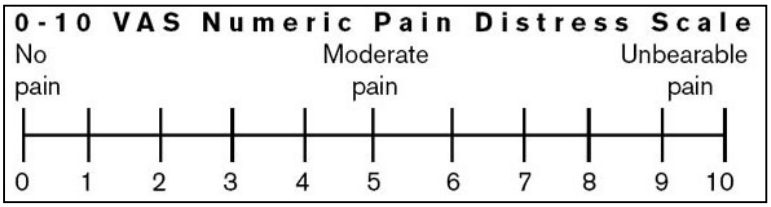

Figure 2.1: VAS Scale utilized during the study where 0 represented no pain and 10 represented highest felt/unbearable pain.

- Time required for walking a distance of 50 feet
- ADL instructions followed or not (only in the last week)
- Quantity of Paracetamol taken every 2 weeks
- Tenderness index scale (0 = No pain, 1 = Describes pain, 2 = Patient winches, 3 = Patient winches and withdraw the affected part, 4 = The patient will not allow the joint to be touched)

2.4 Statistical analysis

Data were organized, tabulated and aggregated using Microsoft excel. Statistical inferences on the differences in efficacy were drawn based on the aggregated data.

Results

The tablet 3.1 below represents the baseline data of all 20 participants in the 3 separate groups of the study:

Different baseline characteristics of the group of patients studied						
Variables	**Group A**		**Group B**		**Group C**	
Age	48.65±6.60		54.00±9.59		53.05±7.34	
Sex	Male	Female	Male	Female	Male	Female
	3	17	8	12	7	13
Height (cm)	152.25±6.83		155.75±7.06		153.65±5.58	
Weight (kg)	64.95±10.10		63.40±13.00		57.95±9.45	
Body mass index	27.98±3.64		26.01±4.46		24.44±3.05	
Systolic Blood pressure	118.25±14.07		111.50±12.99		111.00±15.01	
Diastolic blood pressure	79.25±9.36		74.75±9.39		76.45±9.82	
Pulse rate per minute	76.42±6.47		84.63±12.17		74.80±6.10	
Pain at rest	5.93±1.32		5.90±1.74		5.30±1.98	
Pain while walking	7.10±1.29		7.60±1.19		5.55±1.61	
Pain while ascending/descending stairs	8.3±1.13		8.60±0.88		7.00±1.38	
Time required for walking a distance of 50 feet	24.70±4.95		23.50±4.62		21.52±3.42	

No significant differences were found between the two groups in any demographic or physiological factors. Amongst the 3 groups of 60 patients, 6 patients of Group C (Paracetamol tablets thrice daily) discontinued due to ineffective pain relief.

Characteristics of the group of patients

Age of patients

The patients were of well distributed age groups having. All 3 groups had good number of patients within age group 40-50 as well as above 50.

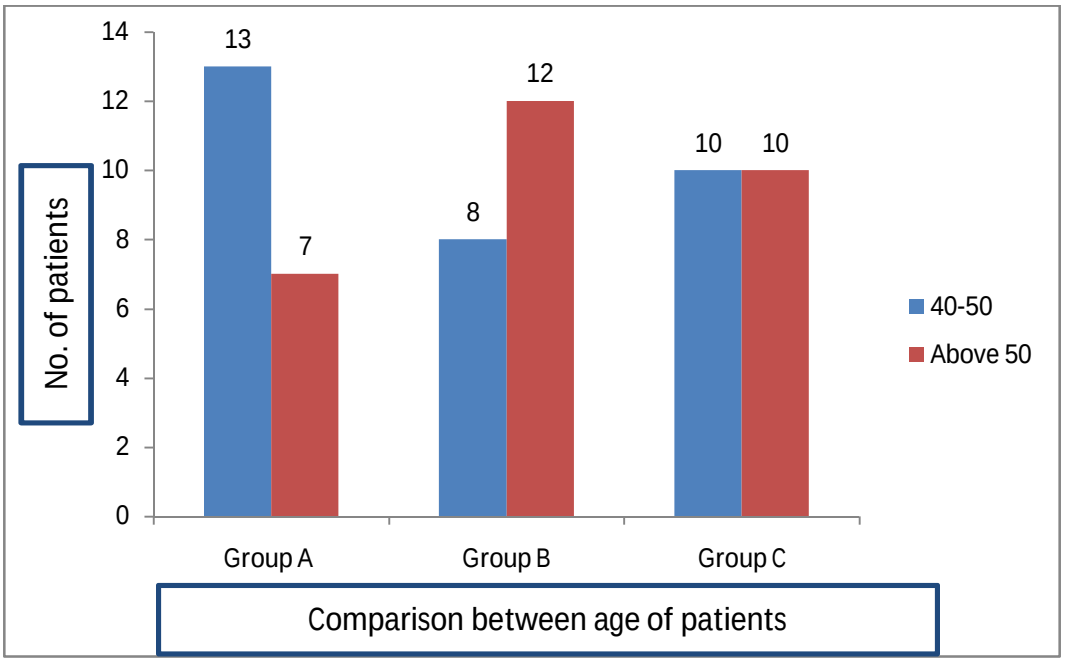

Figure 3.1: Comparison of patients of different age groups within the 3 different treatment groups

Sex of patients

In all 3 groups, majority of the patients were females as is shown in the figure below. Which also shows that females are more affected by the disease.

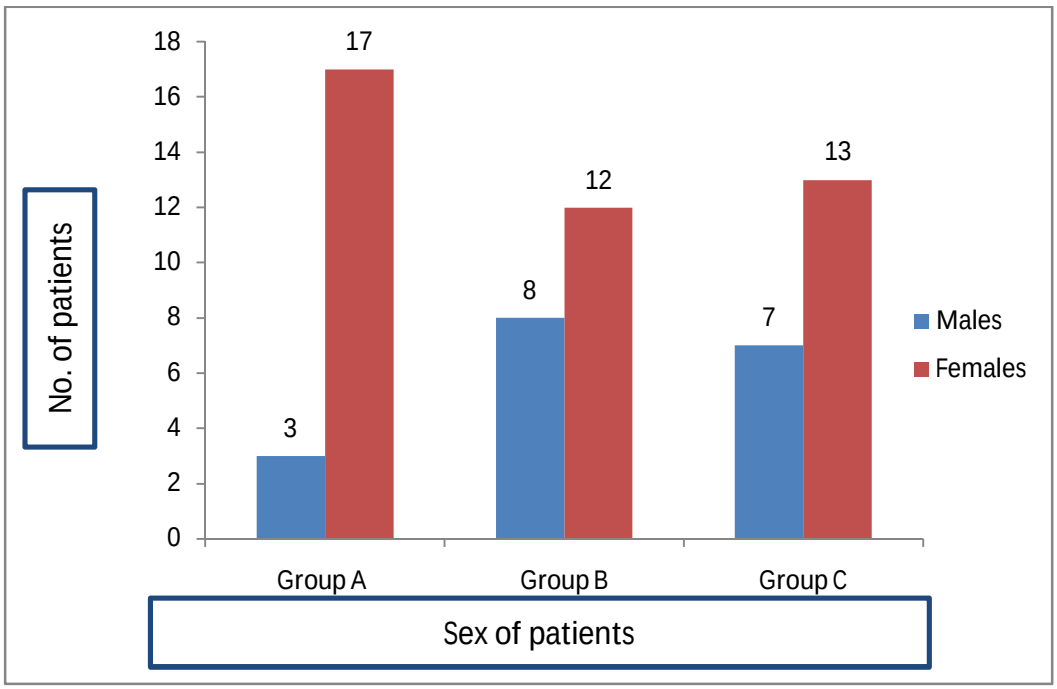

Figure 3.2: Sex of patients in the different treatment groups

Socioeconomic condition of the patients

Majority of the patients were poor. Some of the patients in the study were of middle class.

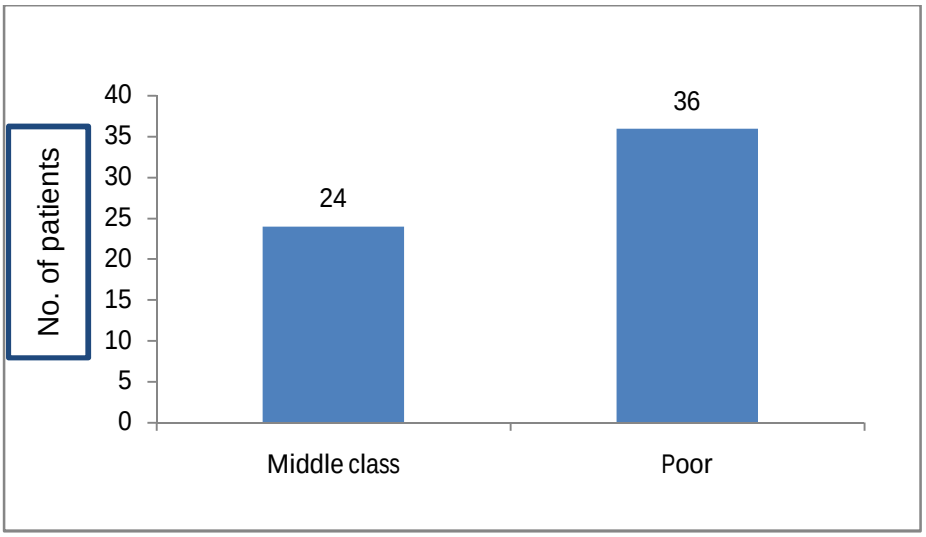

Figure 3.3: Socioeconomic conditions of the patients selected for the study

Religion

Majority of the patients were muslims. This information is particularly important for this study since most of the participants don't pay attention to the ADL instructions and kneel/squat while offering prayers which causes additional pressure on the knees.

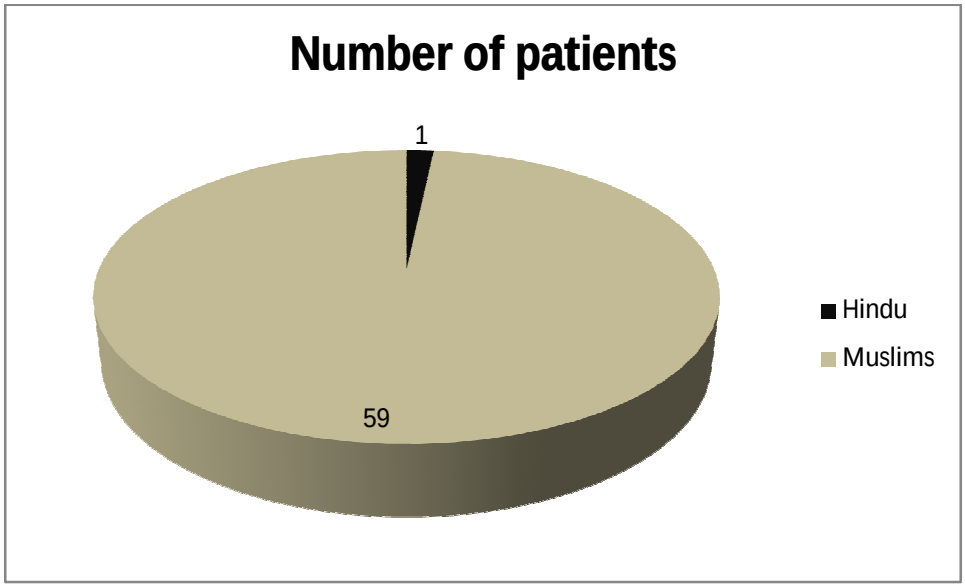

Figure 3.4: Pie chart illustrating the religion of the patients selected for the study

Leg affected

Majority of the patients described their pain to be in the right leg. In group C a higher number of patients had pain in the left leg compared to the other 2 groups.

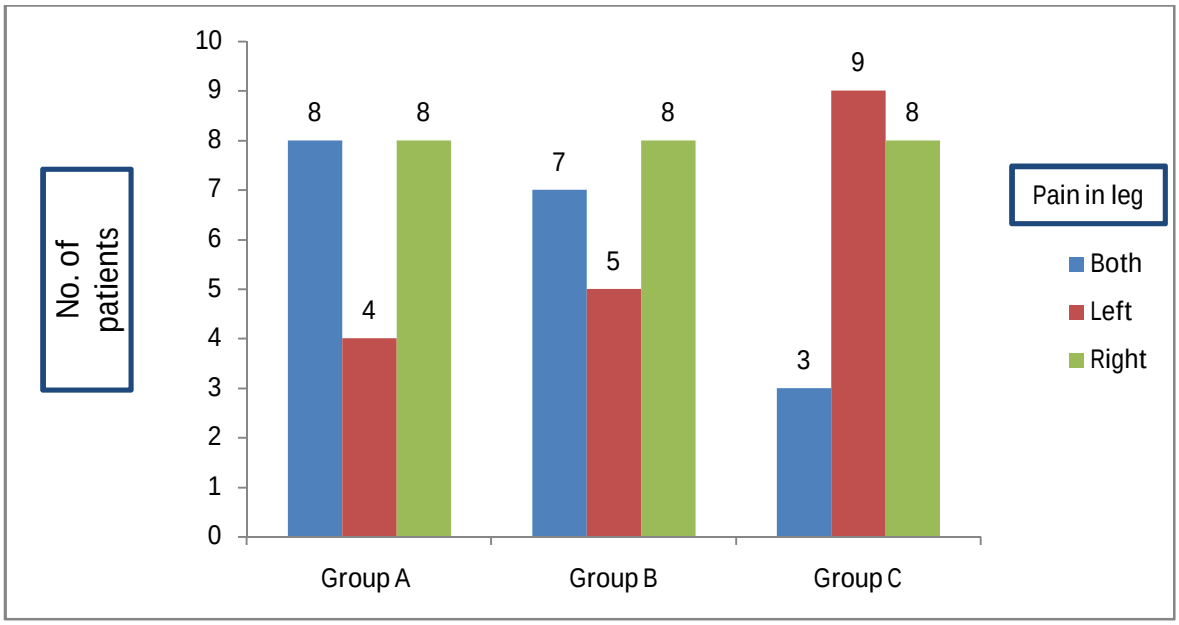

Figure 3.5: Illustration showing the leg in which patients have osteoarthritis in different groups.

Duration of pain

Amongst the selected patients, group A had more patients with duration of pain greater than one year. While group B and C had greater number of patients having duration of pain less than 1 year. The differences in duration of pain can be further used to differentiate in reduction in pain walking times among the different treatment groups.

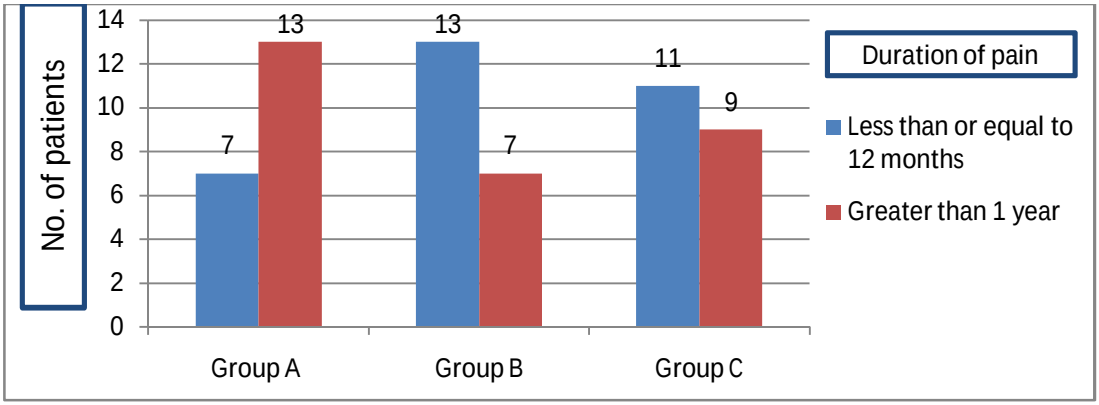

Figure 3.6: Duration of pain amongst the 3 study groups

Onset of pain

In all 3 groups, higher number of the patients had a gradual onset of pain.

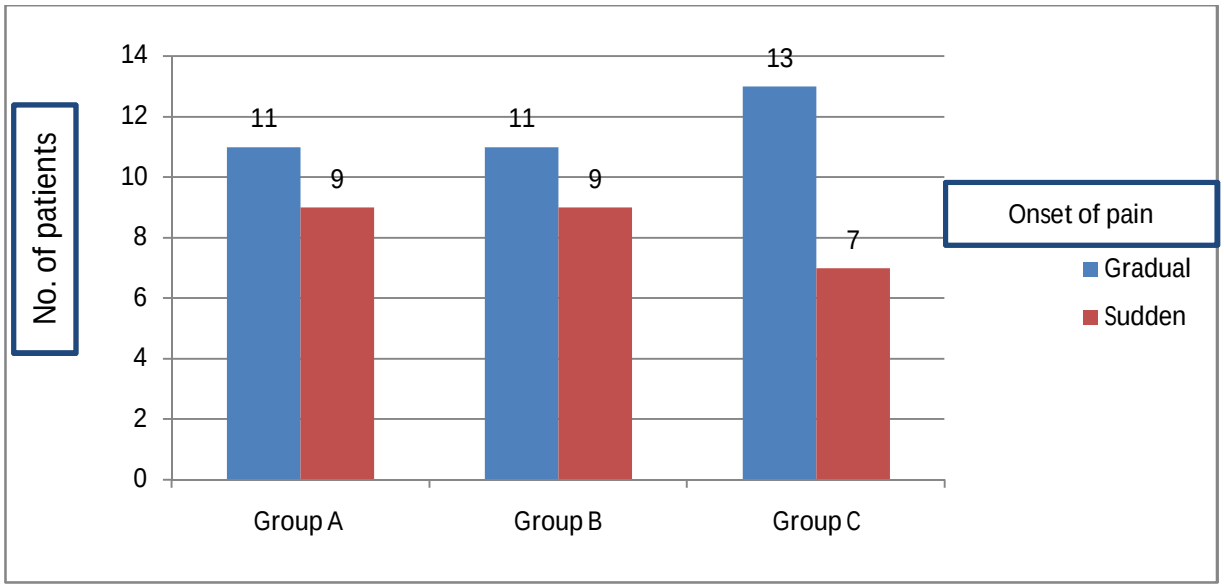

Figure 3.7: Bar diagram illustrating differences in the onset of pain amongst the patients

Time of occurrence

Most of the patients had higher pain occurrences during the morning, evening and night. Some patients had the pain throughout the day.

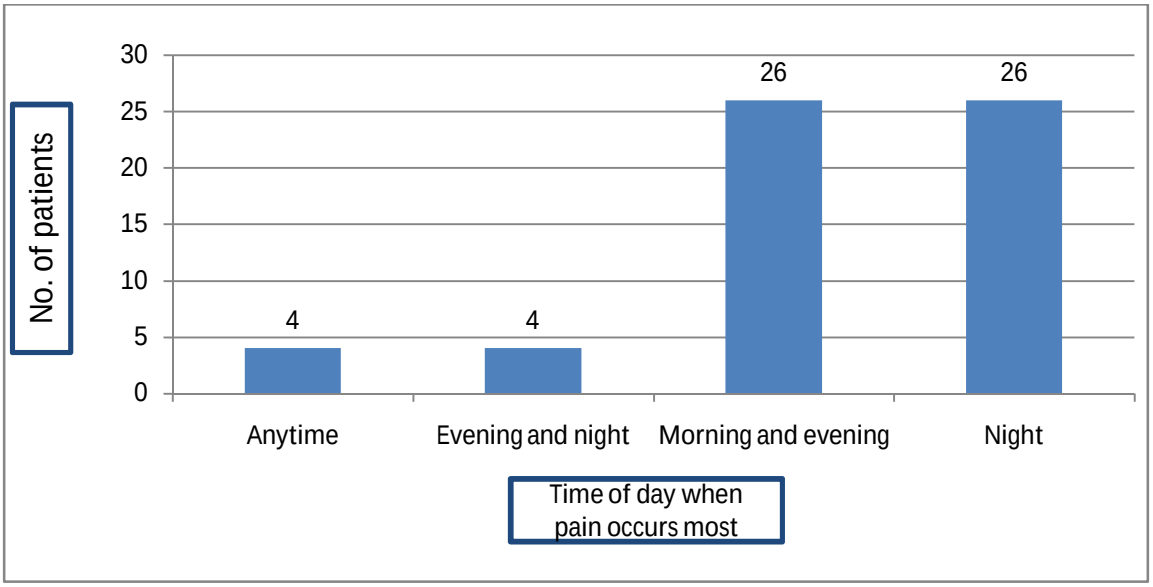

Figure 3.8: Bar diagram showing the different times of the day when pain occurs the most

Characteristics of the pain

Majority of the patients described their pain sensation as sharp while others described it as dull or even stabbing.

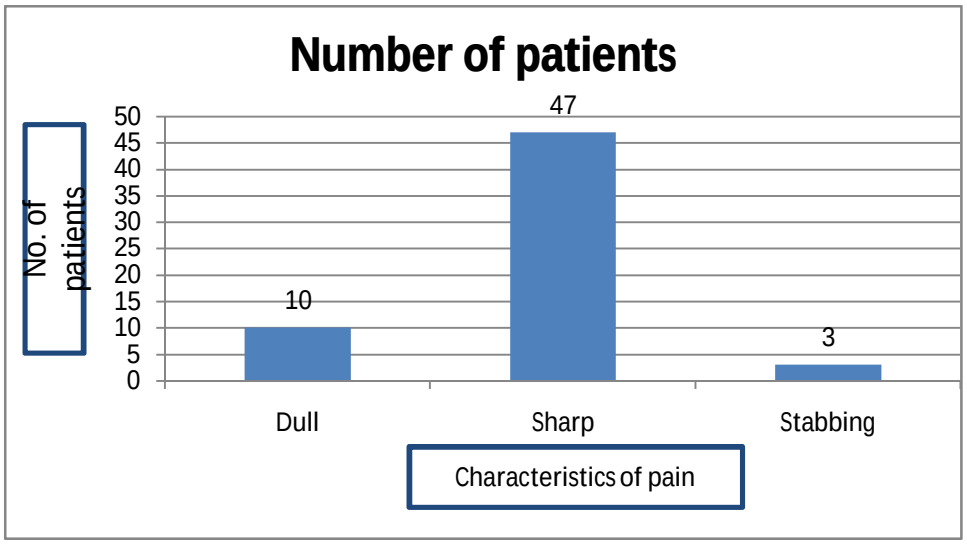

Figure 3.9: Bar diagram illustrating the characteristics of the pain amongst the patients

Aggravating factors

For majority of the patients, the aggravating factors for increasing painful sensation were stair climbing/walking on uneven surface. Some patients also considered prolonged standing or even rest as the aggravating factors.

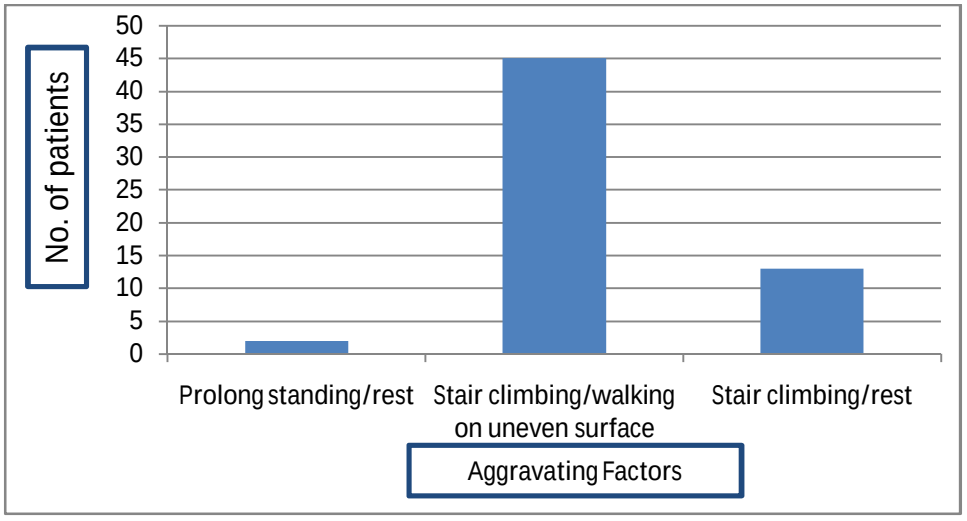

Figure 3.10: Different aggravating factors causing increased pain amongst the selected patients

Pain Relieving factors

Majority of the patients considered rest and heat as the relieving factors, while others considered exercise/activity and heat as the relieving factors.

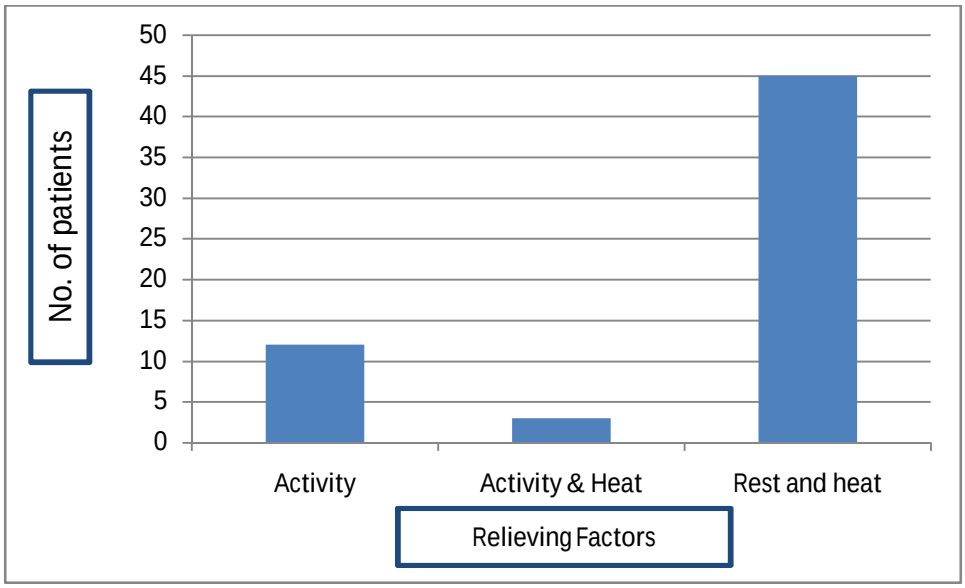

Figure 3.11: Bar diagram illustrating different relieving factors amongst the selected patients

Disabilities

Majority of the patients considered walking as their disability due to pain. There were others who also considered squatting as their disability. Some of the patients had no disabilities.

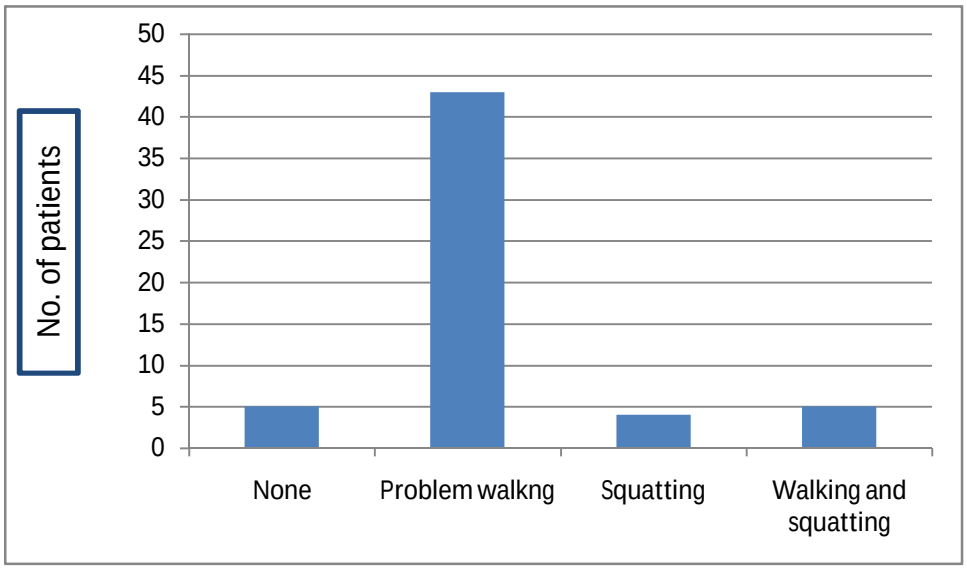

Figure 3.12: Different disabilities amongst the patients in the study

Treatment history

Majority of the patients had already taken NSAIDS and/or exercise for their pain, suggesting that physicians rely heavily on these modes of treatment for knee osteoarthritis.

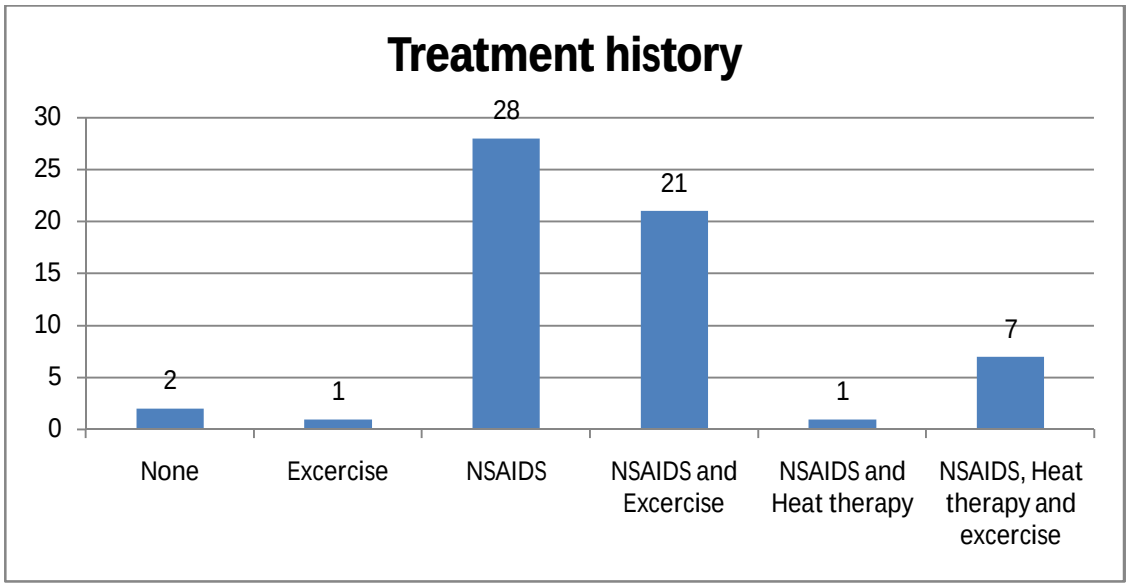

Figure 3.13: Treatment history of the patients participating in the study

Comparison of BMI amongst selected patients

Majority of the patients in the 3 groups were found to be within the overweight (40%) or obese category (25%).

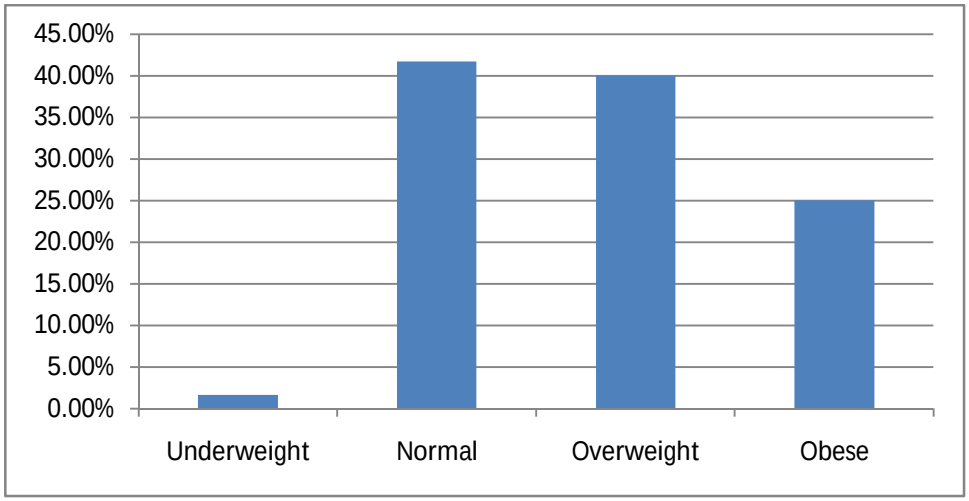

Figure 3.14: Percentage of patients falling within different BMI Categories. Underweight: 18.4 or below, Normal: 18.5-24.9, Overweight: 25.0-29.9, Obese: 30 and above

The normal, overweight and obese patients are well distributed in the 3 treatment groups and therefore can be used for further comparison.

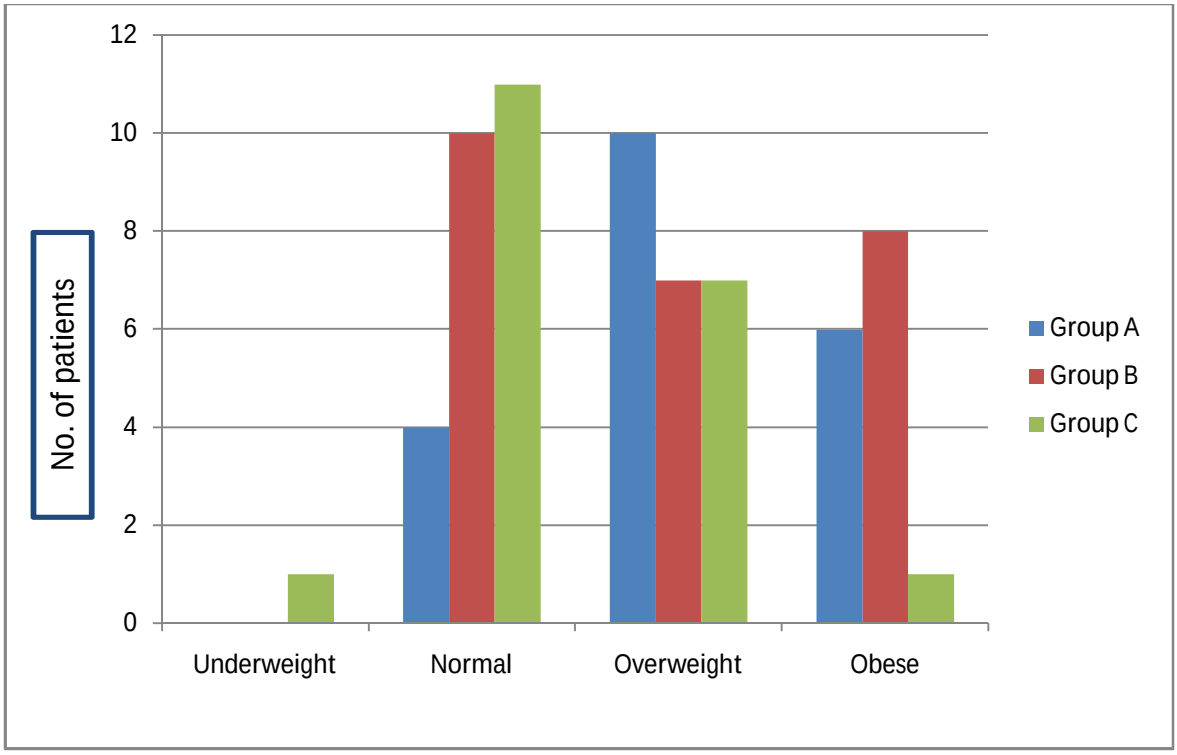

Figure 3.15: Comparison of BMI amongst patients participating in the study. Underweight: 18.4 or below, Normal: 18.5-24.9, Overweight: 25.0-29.9, Obese: 30 and above

Clinical diagnosis

Majority of the patients were diagnosed with Tibiofemoral osteoarthritis on one or both legs. Very few were diagnosed with patellofemoral osteoarthritis on one leg.

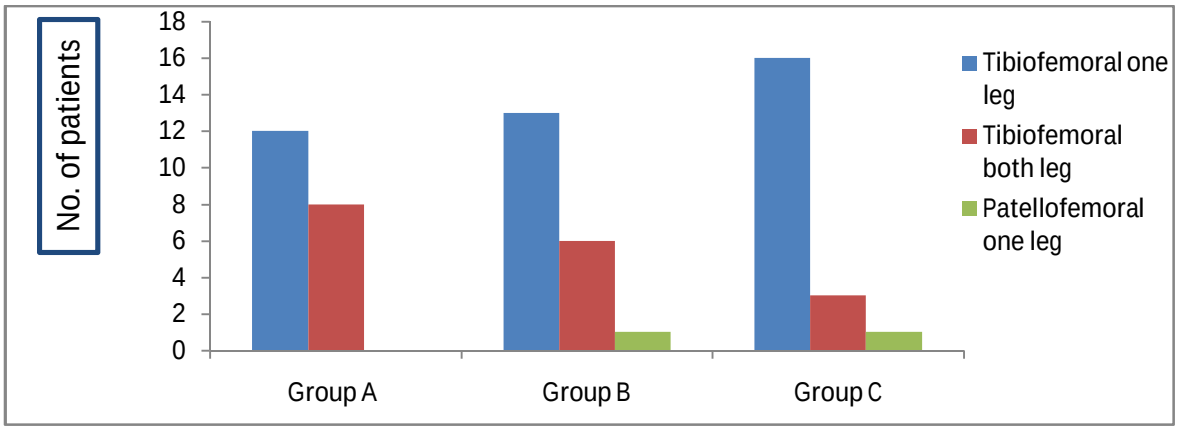

Figure 3.16: Diagnosis of different types of osteoarthritis amongst selected patients

Evaluation of reduction in pain from baseline

Reduction of pain from baseline at rest

The average pain at rest of the patients reduced least amongst patients of group C on every subsequent week from baseline. Whereas, both Group A and Group B showed significant reduction on pain with Group A showing highest reduction from baseline.

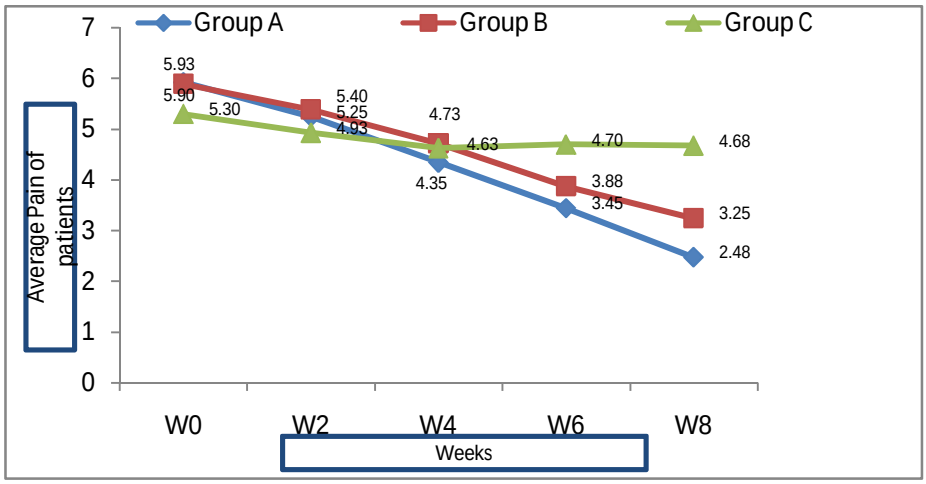

Figure 3.17: Reduction of average pain amongst the 3 groups from baseline

Group A resulted in the highest reduction of average pain (58.18%) after the period of 8 weeks with group C had a maximum reduction of 12.64% at Week 4 after that the average pain reduction of the patients remained fairly similar for the subsequent weeks.

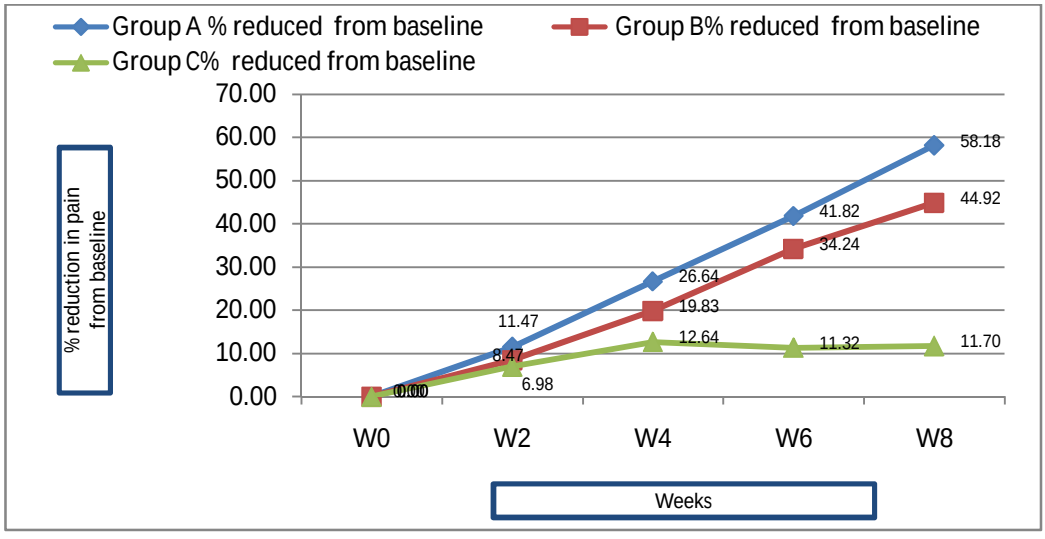

Figure 3.18: Percentage reduction of average pain at rest from baseline amongst the 3 groups of patients

Reduction of pain from baseline during walking

The average pain during walking amongst the patients reduced least amongst patients of group C on every subsequent week from baseline. Whereas, both Group A and Group B showed significant reduction on pain with Group A showing highest reduction from baseline.

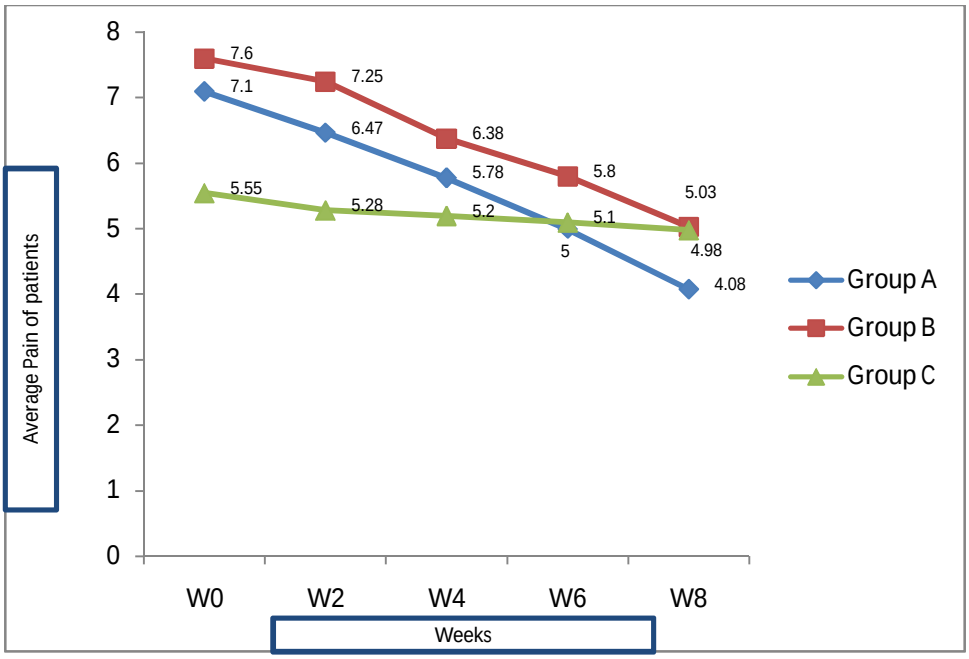

Figure 3.19: Reduction of average pain from baseline amongst the 3 groups from baseline during walking

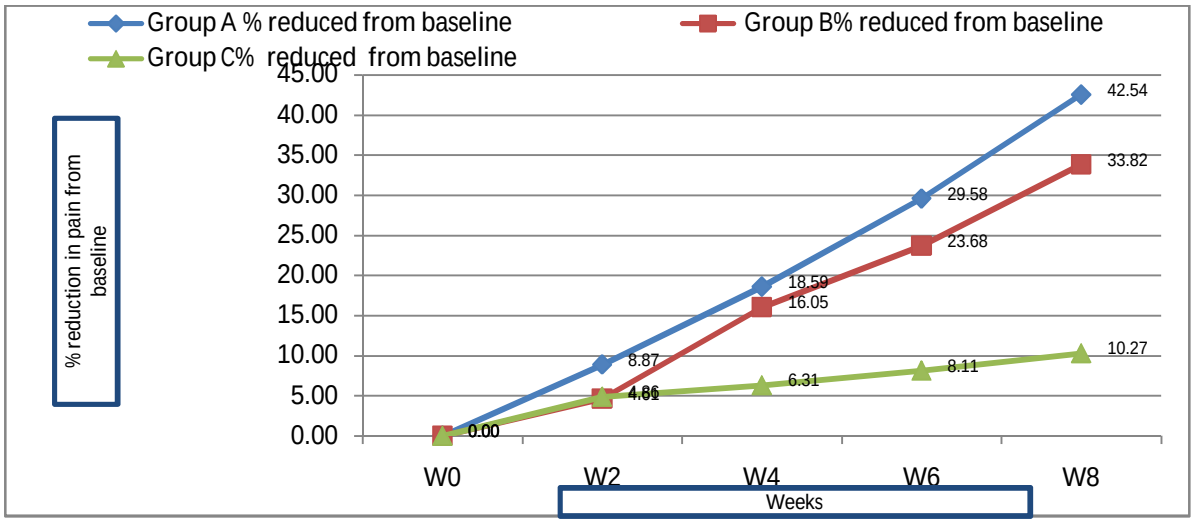

Figure 3.20: % Reduction of average pain amongst the 3 groups from baseline during walking

Reduction of pain from baseline in ascending/descending stairs

The average pain during ascending/descending stairs amongst the patients reduced least amongst patients of group C on every subsequent week from baseline. Whereas, both Group A and Group B showed significant reduction on pain with Group A showing highest reduction from baseline.

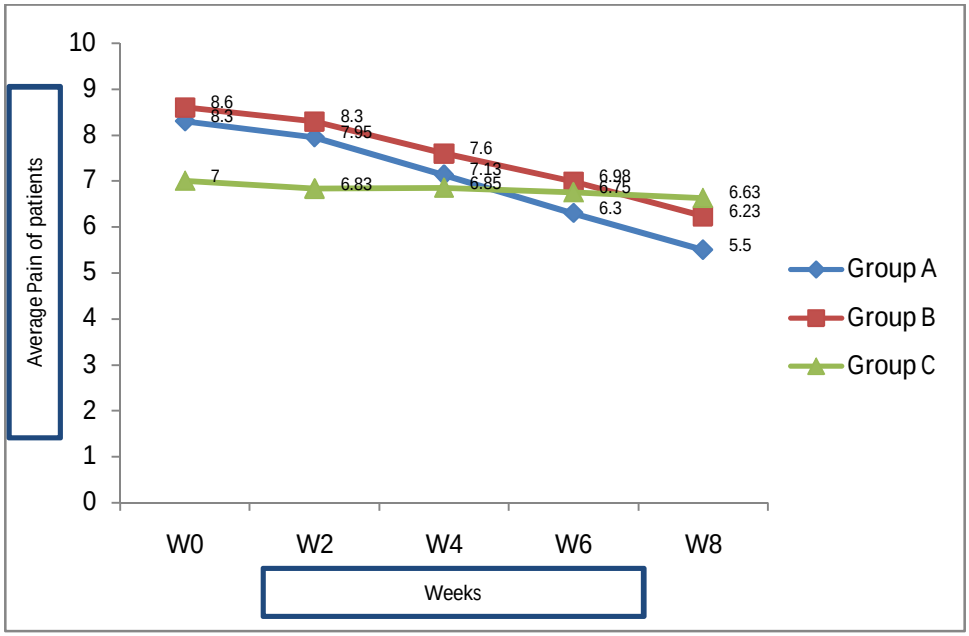

Figure 3.21: Reduction of average pain amongst the 3 groups from baseline in ascending/descending stairs

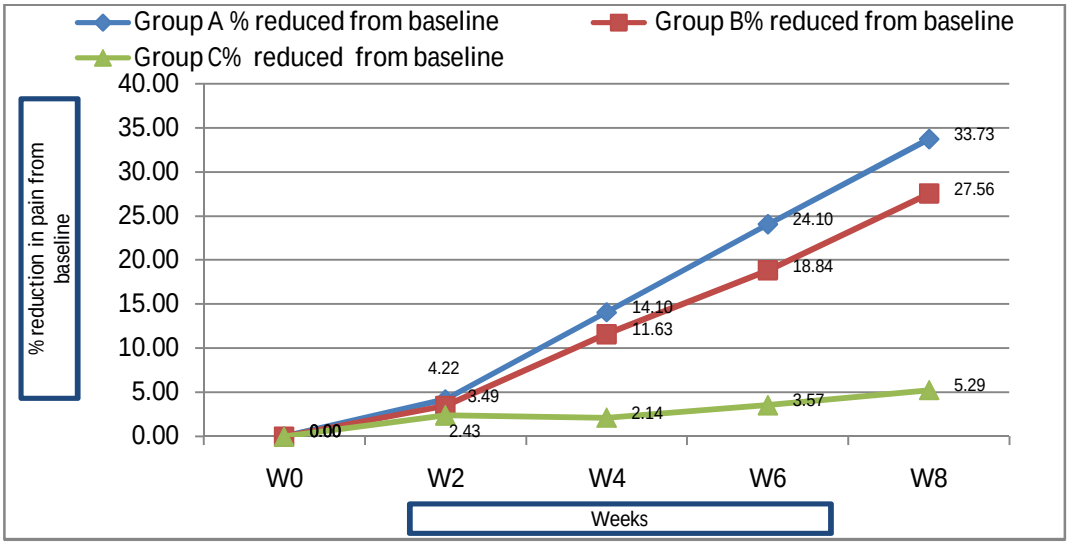

Figure 3.22: Percentage Reduction of average pain from baseline amongst the 3 groups in ascending/descending stairs

Reduction in walking time from baseline

Group C showed least reduction in walking time after a period of 8 weeks, whereas Group A and B showed similar reductions in walking time after 8 weeks.

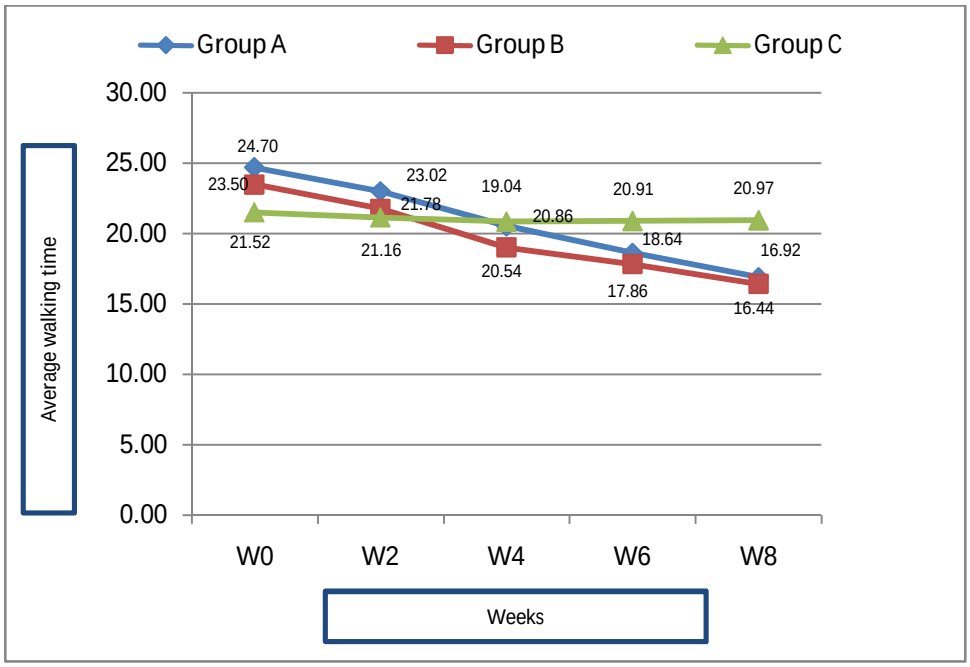

Figure 3.23: Reduction of walking time from baseline amongst the 3 groups from baseline

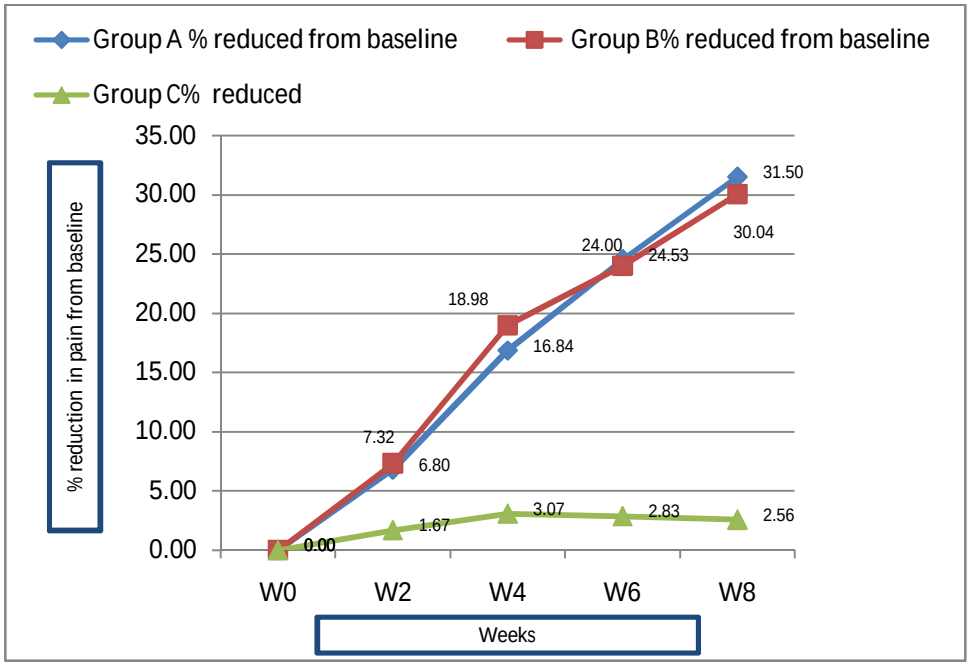

Figure 3.24: Percentage Reduction of walking time from baseline amongst the 3 groups from baseline

Quantity of analgesics taken

Group A and B showed the highest reduction in analgesics taken by the patients after the 8 week study period (48% and 49% respectively). Group C had the lowest reduction in analgesics taken.

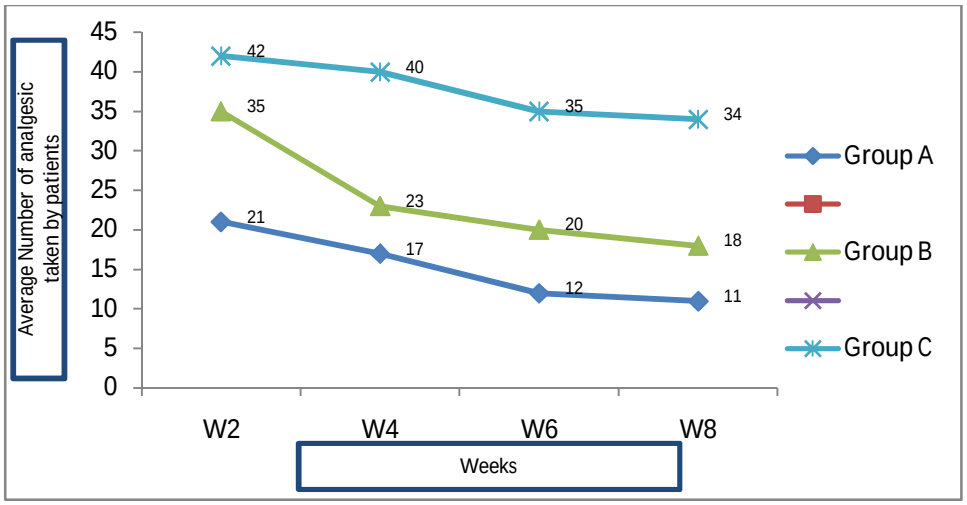

Figure 3.25: Average quantity of analgesics taken by the patients of the different groups during the study

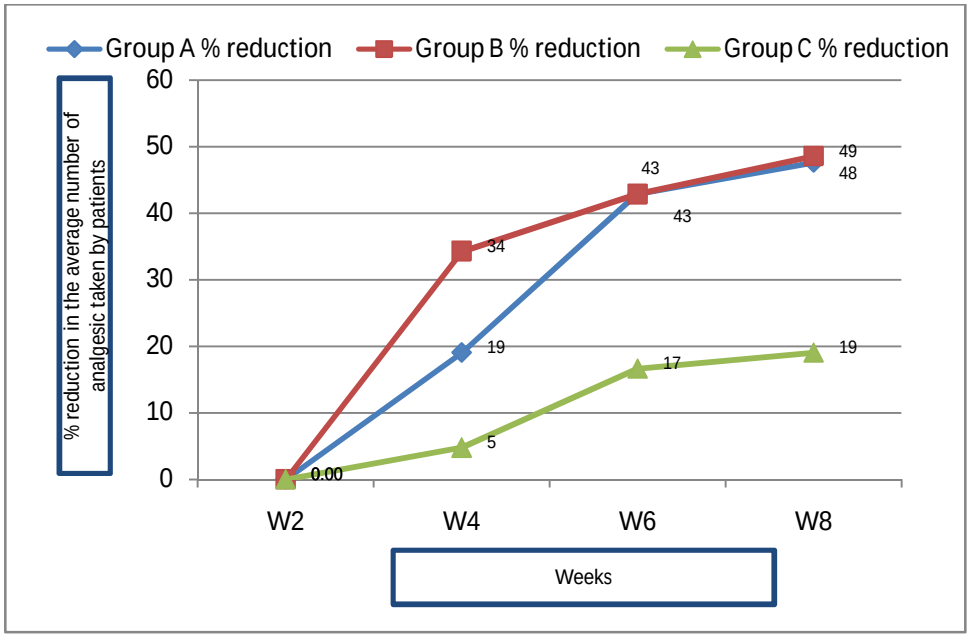

Figure 3.26: Percentage reduction in the average number of analgesics taken by the patients of the different groups during the study

Adherence to ADL (Activities of daily living) instructions

During the study period, the patients were asked whether they were following the different ADL. The results were as given in the figure below. Most patients followed ADL instructions as recommended. However, many patients only partially followed it. i.e. Even though they exercised as recommended, they kneeled during prayers, sat in a squatting position in the toilet, etc. which placed extra pressure on the knees. Some patients did not even exercise as prescribed.

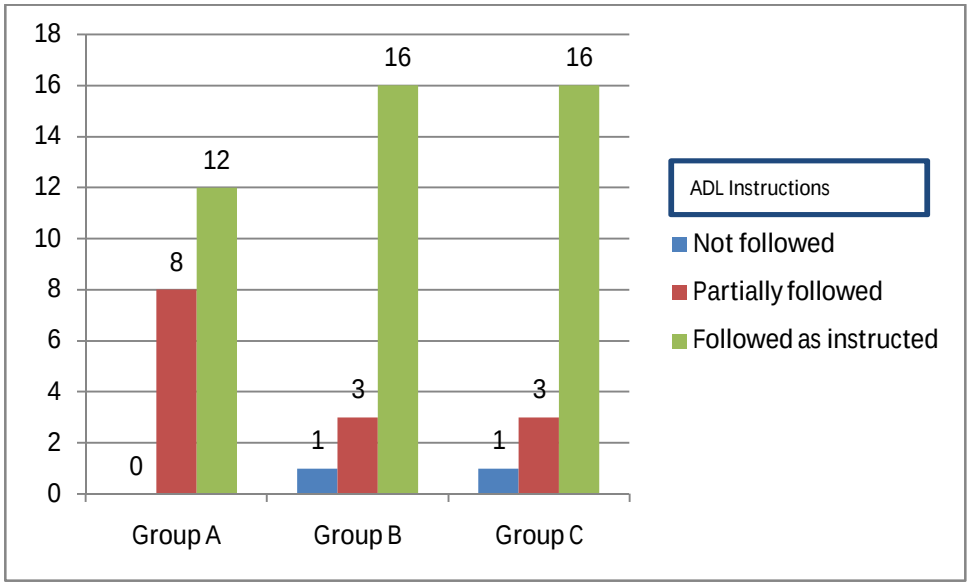

Figure 3.27: Comparison between adherence to ADL instructions amongst the 3 groups of patients

Efficacy of different treatment groups in mild, moderate and severe pain

In order to understand the efficacy of the treatment groups, the VAS pain scale was further categorized into the following. Pain sub scale 1-3 considered as low pain, pain subscale of 4-7 considered as moderate pain, while pain sub scale of 8-10 considered as severe pain. Further analysis was not done on the low pain subscale since very few patients were within that group.

Comparison between % Reduction of average pain from baseline amongst patients having moderate and severe pain at rest

When comparing the % reduction in average pain amongst patients having moderate or severe pain at rest, it was found that all 3 groups showed better efficacy amongst patients having moderate pain, rather than severe pain. In both cases group A had the highest reduction of pain from the baseline 66.30% in moderate pain and 36.36% in severe pain. Group C showed a negative trend in severe pain reduction after 6th week indicating that it was ineffective in reducing pain of the group of patients.

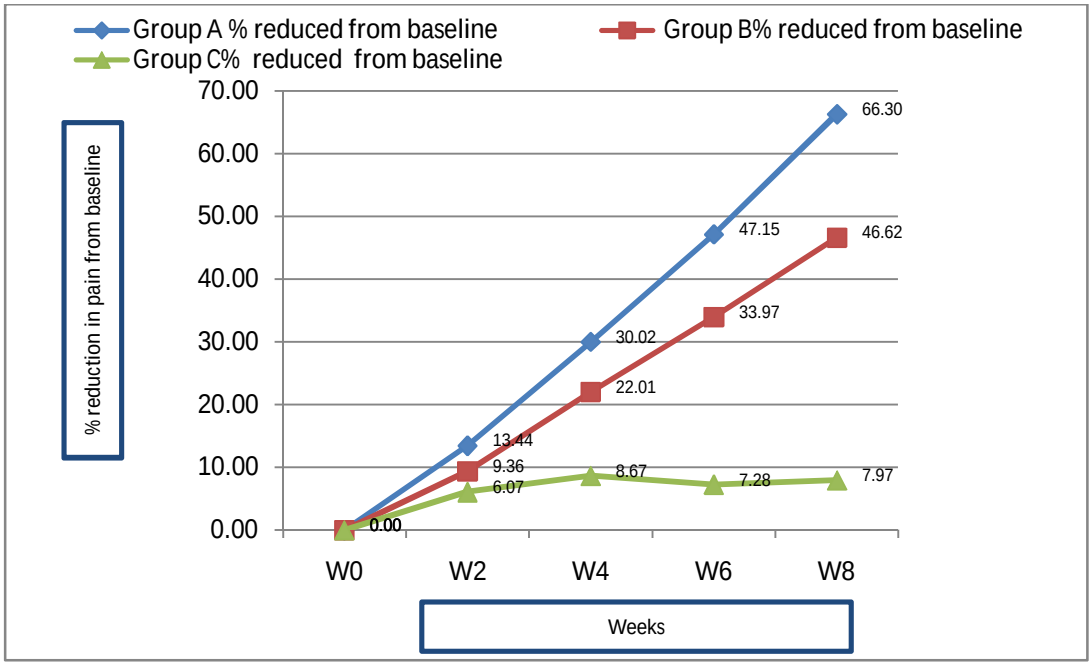

Figure 3.28: Percentage reduction in average pain amongst patients having moderate pain at rest

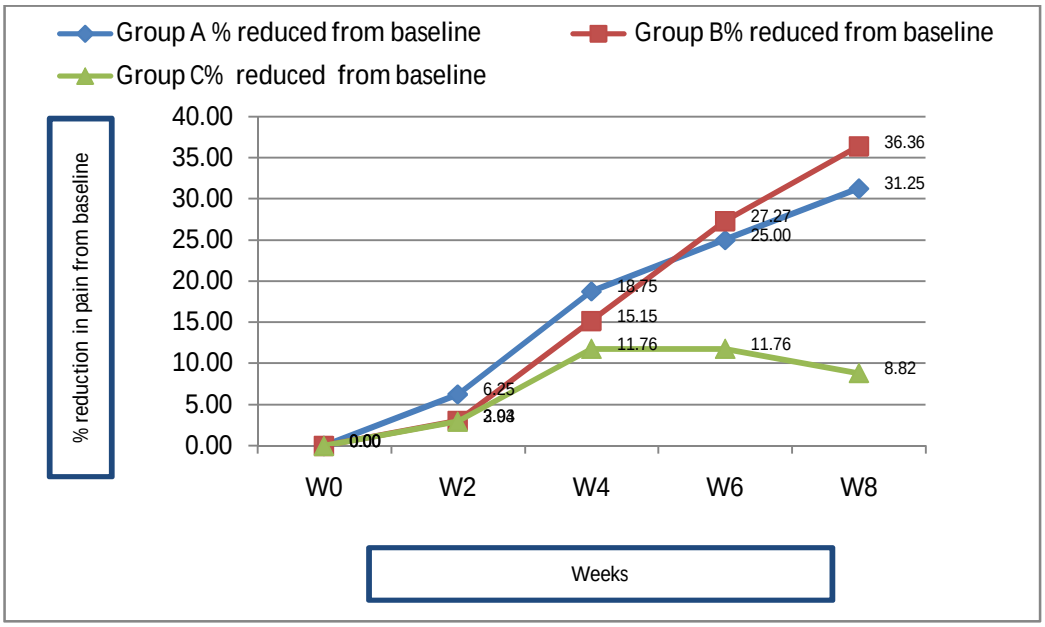

Figure 3.29: Percentage reduction in average pain amongst patients having severe pain at rest

Comparison between % Reduction of average pain from baseline amongst patients having moderate and severe pain while walking

When comparing the % reduction in average pain amongst patients having moderate or severe pain while walking, it was found that all 3 groups showed better efficacy amongst patients having moderate pain, rather than severe pain. Group A had the highest reduction of pain from the baseline 50.98% in moderate pain and 33.54% in severe pain. Group C showed a negative trend in severe pain reduction after 2nd week indicating that it was ineffective in reducing pain of the group of patients.

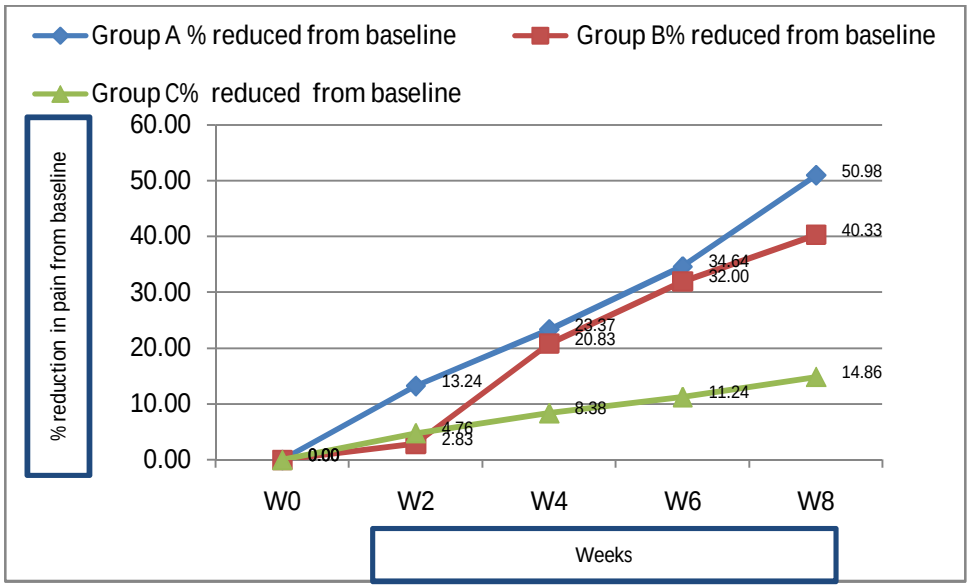

Figure 3.30: Percentage reduction in average pain amongst patients having moderate pain while walking

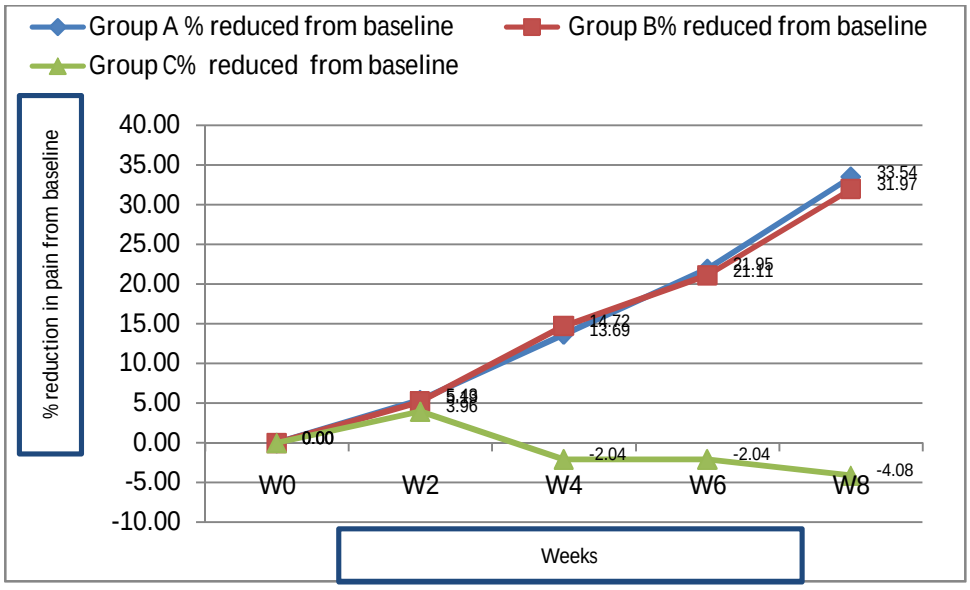

Figure 3.31: Percentage reduction in average pain amongst patients having severe pain while walking

Comparison between % Reduction of average pain from baseline amongst patients having moderate and severe pain while walking

When comparing the % reduction in average pain amongst patients having moderate or severe pain while ascending/descending stairs, it was found that groups A and B showed better efficacy amongst patients having moderate pain, rather than severe pain. Group C had no patients with moderate pain in this category. Group A & B had the highest reduction of pain from the baseline 33.29% in moderate pain while Group A showed highest reduction in average pain in severe pain 31.49% . Group C showed a negative trend in severe pain reduction after 2nd week indicating that it was ineffective in reducing pain of the group of patients.

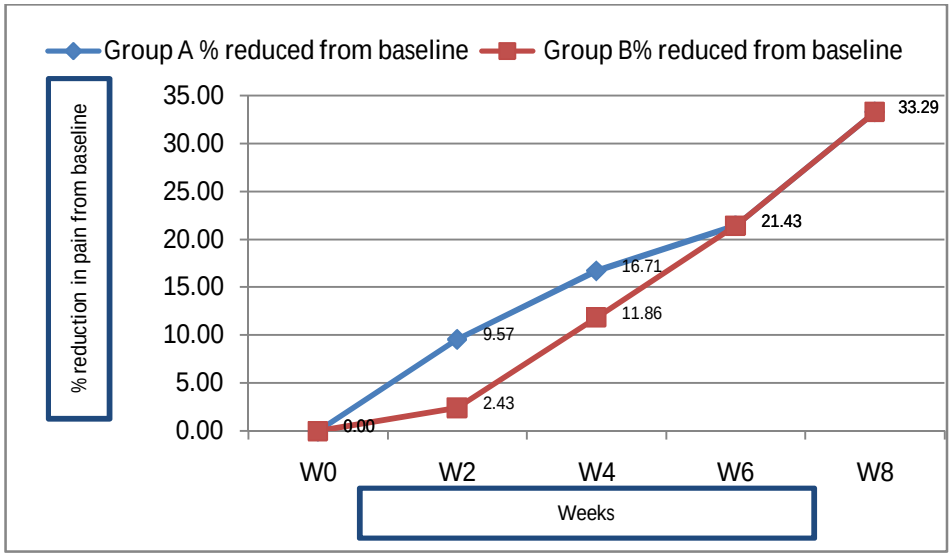

Figure 3.32: Percentage reduction in average pain amongst patients having moderate pain while ascending/descending stairs (Group C had no patients with moderate pain while ascending/descending stairs)

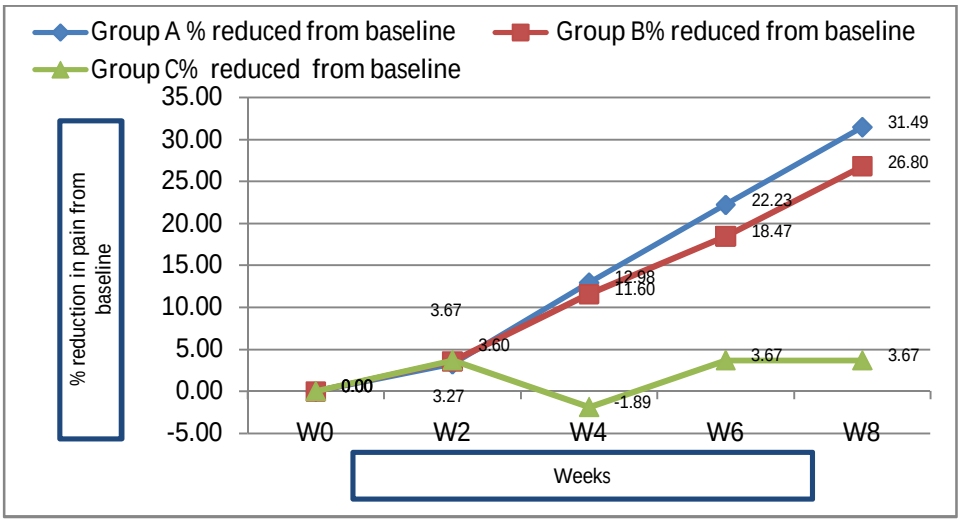

Figure 3.33: Percentage reduction in average pain amongst patients having severe pain while ascending/descending stairs

Comparison between % Reduction of average pain from baseline amongst patients having different BMI

As per accepted standards, the BMI of the patients were categorized as below:

- Underweight: 18.4 or below
- Normal: 18.5-24.9
- Overweight: 25.0-29.9
- Obese: 30 and above

Accordingly the patients that were normal weight, overweight and obese were evaluated for their reduction in average pain as below.

Comparison between reduction of average pain from baseline amongst patients having normal weight, overweight and obese

On an average the obese patients had a higher average pain at baseline level compared to patients having normal weight. However, no significant differences were seen amongst patients having normal weight, overweight or obese patients.

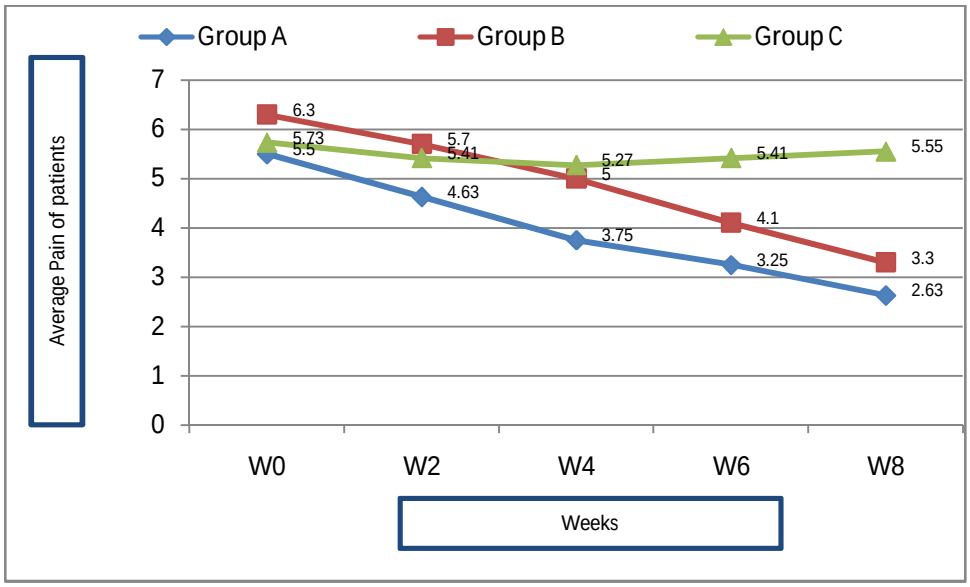

Figure 3.34: Reduction in average pain at rest amongst patients having normal weight

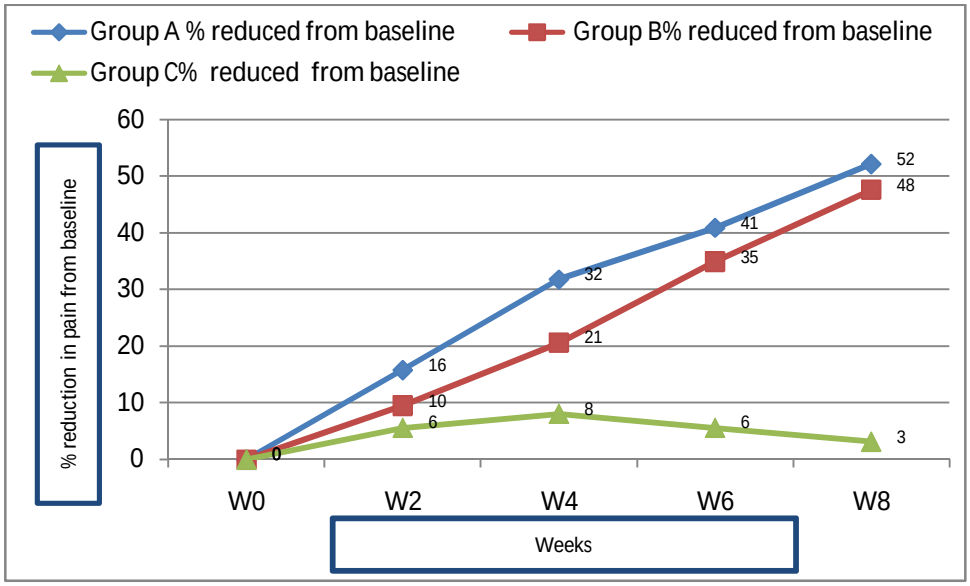

Figure 3.35: Percentage reduction in average pain at rest amongst patients having normal weight

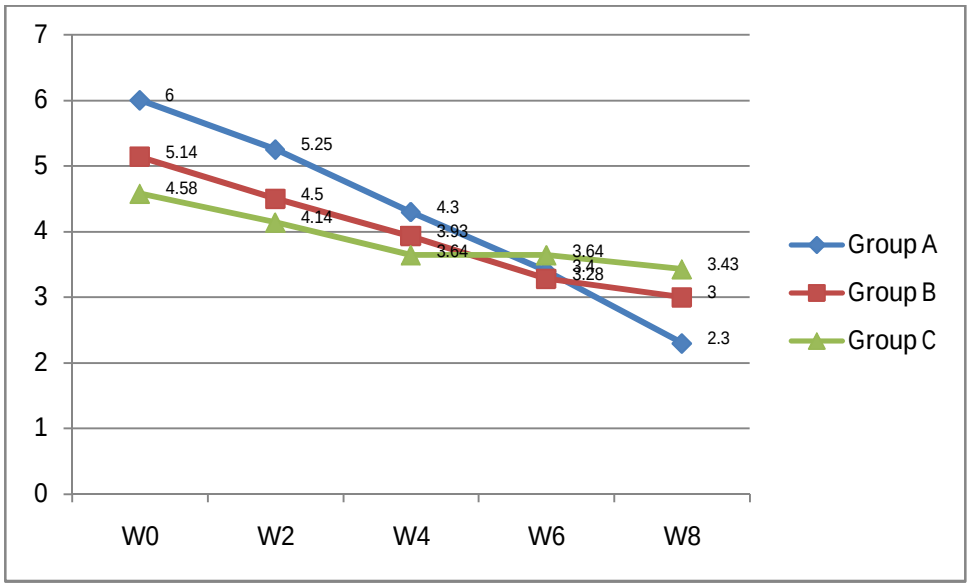

Figure 3.36: Reduction in average pain at rest amongst overweight patients

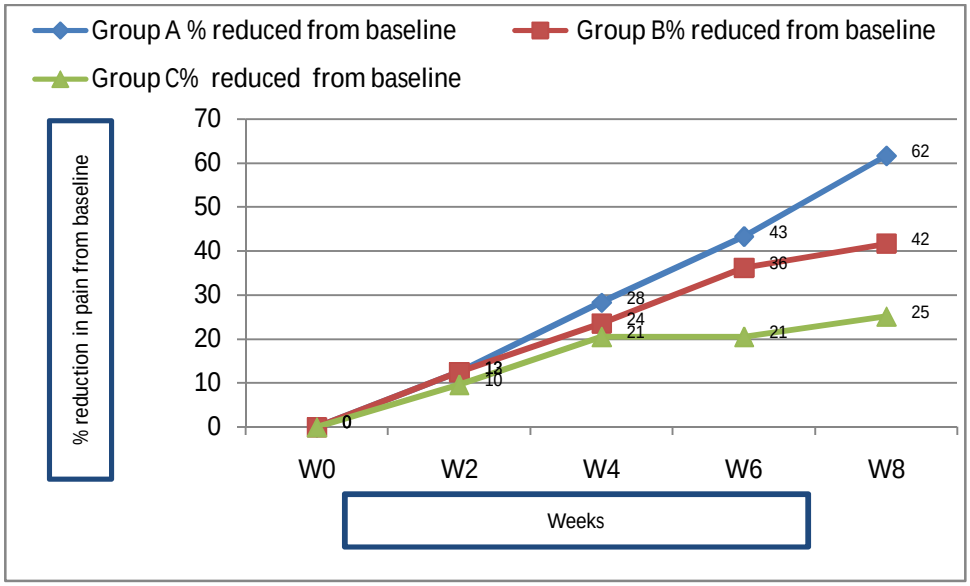

Figure 3.37: Percentage reduction in average pain at rest amongst overweight patients

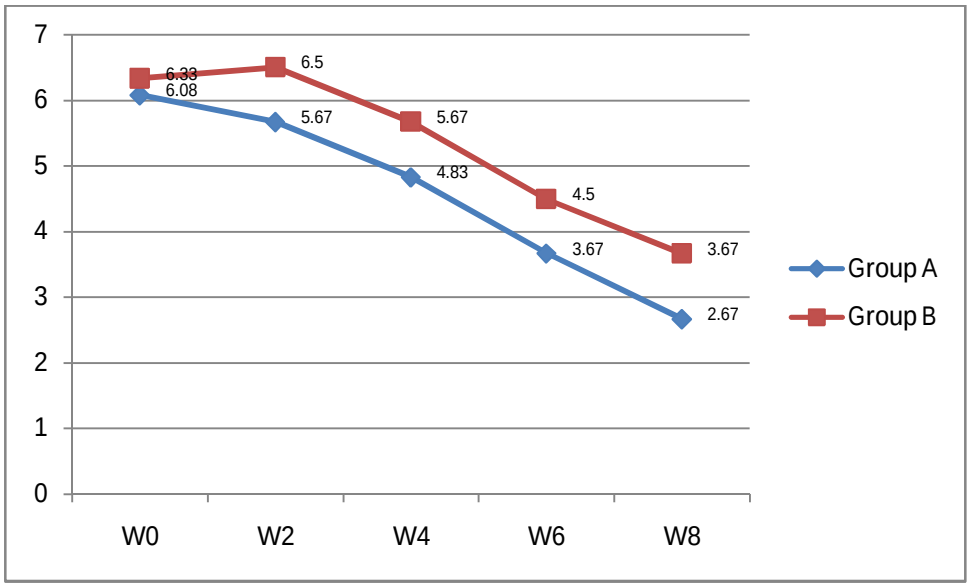

Figure 3.38: Reduction in average pain at rest amongst obese patients

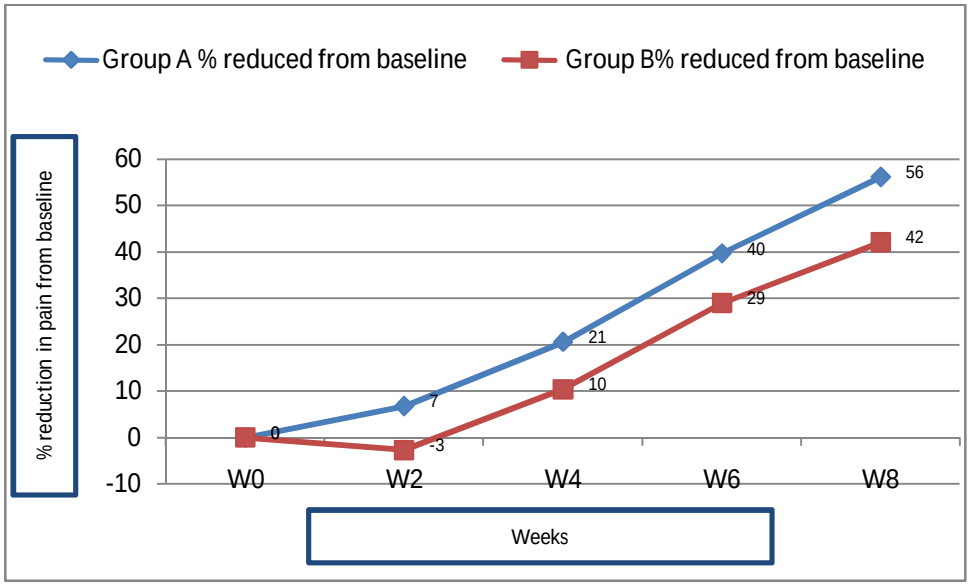

Figure 3.39: Percentage reduction in average pain at rest amongst obese patients

Tenderness Index scale

The tenderness index scale yielded insignificant results. The average was more or less the same upto 8 weeks. As a result the data has not been used to draw any further inferences.

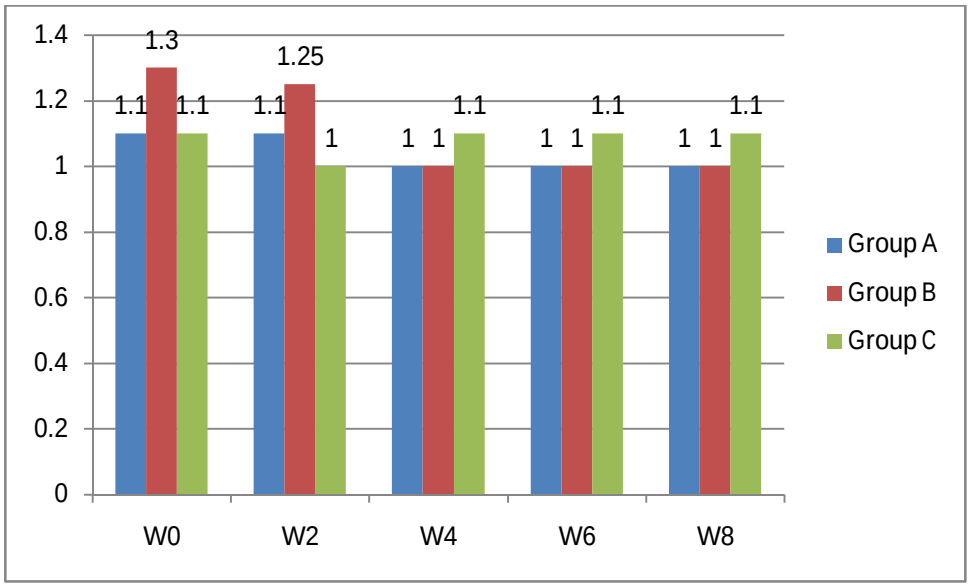

Figure 3.40: Tenderness index scales of the 3 groups of patients

Discussions

The prospective, randomized single blinded clinical trial was carried out to evaluate and differentiate between the effects of the 3 treatment groups below:

- Group A: Glucosamine sulphate + Chrondoitin sulphate + Vitamin C + Paracetamol
- Group B: Glucosamine sulphate + Chrondoitin sulphate + Paracetamol
- Group C: Paracetamol

Even though extensive studies have been conducted on efficacy of glucosamine and chrondoitin sulphate, in most of the studies, the combination were always given alone without any analgesics. However, as per a number of previous studies it has been shown that glucosamine sulphate and chrondoitin sulphate is not appropriate for short-term analgesia, but is suitable for medium- to long-term management of knee OA, producing global clinical improvements.[61,94] Ascorbic acid stimulates collagen synthesis and modestly stimulates synthesis of aggrecan (a proteoglycan present in articular cartilage). Sulfated proteoglycan biosynthesis is significantly increased in the presence of ascorbic acid.[69]

Therefore, in this study the outcome of combining the analgesic with combination of glucosamine sulphate, chrondoitin sulphate and vitamin C was checked.

The results of the of the study show that the combination treatment of Group A (Chrondoitin sulphate + Glucosamine + paracetamol) was much more superior in reduction of pain from baseline when compared to the other 2 groups (Group B & C) at rest, while walking or even ascending/descending stairs amongst the patients.

The percentage reduction of average pain from baseline for Group A at rest was 58.18% after 8 weeks. Whereas, the percentage reduction of average pain of Group B and Group C were 44.92% and 11.70% respectively.

The percentage reduction of average pain from baseline for Group A while walking was 42.54% after 8 weeks. Whereas, the percentage reduction of average pain of were 33.83% and 10.27% respectively.

The percentage reduction of average pain from baseline for Group A at rest was 33.73% after 8 weeks. Whereas, the percentage reduction of average pain of were 27.56% and 5.29% respectively.

This shows that the effectiveness of all 3 treatment modalities decreases as a whole when at rest compared with while walking or climbing stairs. As expected pain reduction

was lowest in Group C who were given Paracetamol which is only effective in mild OA.[48,49] On the other hand the the superior reduction in pain Group A compared to Group B maybe attributed to combined effects of vitamin C, chrondoitin sulphate and glucosamine. In vitro studies have shown that glucosamine enhances the production of cartilage matrix components in chondrocyte culture, such as aggrecan and collagen type II.[56,57] Glucosamine increases hyaluronic acid production in synovium explants. Further experiments have shown that glucosamine prevents collagen degeneration in chondrocytes by inhibiting lipoxidation reactions and protein oxidation.[58] The negative charge of Chrondoitin Sulphate, makes it responsible for the water retention of the cartilage, which is important for pressure resistance. [61] Both of these nutrients were also found to modulate the inflammatory process and act as antioxidants reducing oxidative stress. Both of these functions are beneficial in OA.[65,66,67] Vitamin C is a very good antioxidant. In studies involving animals protective effect on experimentally induced cartilage degeneration of the knee.[71,72] These effects have been also demonstrated in humans in the Framingham study.[73]

The results of the reduction from baseline has been further reinforced by the reduction in the quantity of analgesics taken by patients after 8 weeks compared to baseline (2nd week) in group A (48%) and Group B (49%) compared to group C (19%).

While comparing the effectiveness of the pain relief in moderate and severe pain, it was found that groups A and B treatments were better in reduction of average pain from baseline inpatients having moderate pain while at rest or walking. Group A treatment was superior to other 2 groups in all cases, except for average reduction in moderate pain of patients while ascending/descending stairs. These findings are inline with previous study where glucosamine sulphate and chrondoitin sulphate were found to be effective in moderate osteoarthritis.[95]

While comparing the average time taken for walking 50 feet distance, it was found that treatment groups A and B yielded almost similar results after 8 weeks (approx. 30-31% reduction from baseline), while group C had the least reduction (2.56%)

All patients were encouraged to follow the ADL instructions besides their daily medications on every fortnightly follow up. On the last week the patients were asked the whether they were able to follow the ADL instructions. It was found that 14 out of 60 patients followed the ADL instructions partially, while 2 out of 60 patients did not follow the ADL instructions at all. The main reasons for not completely following the instructions were the following:

- Many patients took ADL instructions lightly (i.e. they put pressure on their knee joints by getting into squatting positions or kneeling).
- Majority of the patients were poor and had low toilets in their households that requires squatting while using the toilet.
- Approximately 51% of the patients were housewives who usually work in the kitchen by sitting in squatting position.
- Majority of the patients were muslims who have to offer their daily prayers by kneeling/bending legs.

The effects of not following the ADL instructions is not within the scope of this study However, it should be studied extensively on a larger group of patients for a longer period of time.[14,15,16,17]

Even though none of a larger number of randomized clinical trials gave positive results, suggesting an ambiguity of the benefit of the two nutraceuticals glucosamine sulphate and chrondoitin sulfate in OA.[78] Most of the trials compared the effects of glucosamine sulphate and chrondoitin sulphate with NSAIDS which is inappropriate.

The outcome of the treatment with glucosamine and chrondoitin sulphate will always be inferior compared to NSAIDS for short term pain relief. Glucosamine and chrondoitin sulphate are more effective for long term relief of pain with lasting effects even after completion of treatment as per a number of studies. [94, 95]

NSAIDS are highly relied upon by physicians for short term pain relief in patients with knee OA. [40,41] This fact has been shown in a number of studies and is further reinforced in our study by the fact that 57 out of 60 patients had taken NSAIDS before for their pain relief. Unfortunately, the pain relief achieved from NSAIDS is only for a short term and therefore, patients need to continue taking the NSAIDS to ensure prolonged pain relief. Many NSAIDS are available over the counter which also accounts for its widespread use after prescription. [46,47] After being prescribed due to disability of movement cause by knee osteoarthritis, majority of the patients do not return to the doctor for a follow up but continue taking the NSAIDS as over the counter medicines, since they offer a prompt relief from pain.

This is particularly problematic since long term use of NSAIDS is associated with many adverse events including the destruction of articular cartilage thereby accelerating OA, the disease for which they are so commonly prescribed.[96]

This study has a number of limitations:

- Sample size was small

- Duration of study is small

Therefore, a larger study comprising a bigger sample size for a longer duration need to be undertaken in order to further confirm the findings of this study.

Conclusion

Currently, there is no cure to osteoarthritis. Available treatment options are only aimed at alleviating pain and improving functionality of joints. The analgesic Paracetamol/ Acetaminophen tablet is considered as the first line treatment of in reduction of pain in patients with mild Osteoarthritis.

However, due to insufficient reduction in pain in patients with moderate to severe osteoarthritis, physicians rely heavily on NSAIDS for alleviation of OA pain. However, the long range of side effects of NSAIDS discourages the long term use of these medicines. As a result different nutritional supplements are also getting priority for OA treatment. However, clinical study data of these group of medicines are varying.

In our study we have clearly demonstrated the superior effects of combination treatment considering paracetamol, glucosamine sulphate, chrondoitin sulphate and vitamin C over glucosamine sulphate, chrondoitin sulphate and paracetamol.

Since the other treatment options carry a considerable risk of side effects over long term use, this treatment combination maybe a very valuable and safe method of ensuring long term treatment of knee OA with negligible side effects.

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Research Protocol

Research Protocol		
1.	**Relevant Faculty East West University & Dhaka Medical College**	Relevant Discipline: Department of Pharmacy and Physical Medicine and rehabilitation.
2.	**Name of Chief investigator**	REPON KUMER SAHA
3.	**Name of other investigators**	EKRAM AHMED CHOWDHURY SADIA TANZIN
4.	**Address of Correspondence of the chief investigator and contact phone numbers**	Repon Kumer Saha, PhD Department Pharmacy East West University, A/2 Jahurul Islam City, Aftabnagar, Dhaka Mobile: 01756513045 Email: reponsaha@yahoo.com
5.	**Title of Research**	Effect of a dietary supplement containing glucosamine, chrondoitin sulfate & Vitamin C on osteoarthritis of knee joint.
6.	**Summary:**	

Osteoarthritis (OA) of knee has got many treatment options, but no treatment is complete and specific. Conventional pharmacological approaches to symptom management in OA involve nonsteroidal anti-inflammatory drugs, selective cycloxygenase-2 inhibitors, and intra-articular injection of hyaluronan or corticosteroids. However, there are accumulating data showing that any of these pharmaceutical drugs frequently produce insufficient benefits, with an associated risk of untoward side effects.

Glucosamine hydrochloride and chondroitin sulfate are cartilage extracellular matrix components that have been widely used as alternative medicines or nutraceuticals for the management of OA and have been the subject of a huge number of clinical studies for this purpose. Some clinical trials thus far reported support the demonstrated favorable effects of glucosamine and chondroitin sulfate alone or in combination in relieving OA pain. However, none of a larger number of randomized clinical trials gave such positive results, suggesting an

Research Protocol

	ambiguity of the benefit of these two nutraceuticals in OA. Therefore, based on findings of clinical trials in cultured chrondocytes, a prospective, randomized, experimental study will be performed to see the role of glucosamine, chrondoitin sulphate, & Vitamin C (GCC treatment) in the reduction of pain in osteoarthritis. Patients will be blinded for treatment allocation. Among the patients attending at the Department of Physical Medicine in Dhaka Medical College Hospital, sixty patients with Knee OA (according to selection criteria) will be eligible to be included in the study. The patients will be randomly divided into three groups by lottery. Data will be collected from each patients in every two weeks interval.	
7.	**Place of study**:	Department of Physical Medicine, Dhaka Medical College Hospital.
8.	**Study period**:	1st May, 2013 –30th October, 2013.
9.	**Study Design**	Prospective, randomized, experimental, single blinded study.
10.	**Introduction:** Osteoarthritis is the most common form of arthritis and one of the most important causes of long term disability in adults [1]. OA has a worldwide distribution though there is a variation in the prevalence among different ethnic groups and genders. OA mainly affects the elderly population. The prevalence of OA in population older than 60 years of age is more than 50% [2]. Osteoarthritis (OA) is primarily a disease of cartilage as it is characterized by the degradation of hyaline cartilage in the joints [3]. It is believed to be a dynamic disease that reflects the balance between destruction and repair. The destruction processes of cartilage, softening and fibrillation, exposure of the subarticular bone plate, and fragmentation of the subchondral trabeculae, are accompanied by hyperactive new bone formation, osteophytosis and bone remodeling [4]. Osteoarthritis is characterized clinically by pain, swelling of joints and limitation of motion. Pathological disease is characterized by focal erosive lesions, cartilage destruction, subchondral sclerosis, cyst formation and large osteophyte at the margin of the joints [5]. Diagnosis of Osteoarthritis is based on X-ray evidence of joint space narrowing, subchondral sclerosis or osteophyte formation, and symptom of pain in the affected knee on motion or rest plus at least one of the following; tenderness with pressure; crepitus on motion or stiffness, either in morning or after prolonged inactivity [6]. Common complaints in people with knee OA are pain exacerbated by movement or weight bearing, stiffness, swelling and deformity (genu varum or genu valgum), and restricted walking distance. To date, no curative treatment for OA exists. The objectives of management of OA of the	

knee are to relieve pain, maintain or improve mobility, and minimize disability [7]. Treatment options include non-pharmacologic intervention, drug therapy, and surgery**.** In 1995, the American College of Rheumatology (ACR) published guidelines for the treatment of OA knee. These were updated in 2000(ACR 2000), 2003 (ACR 2003) and lastly in 2012 stating that, for mild symptomatic OA, treatment may include non-pharmacologic methods (patient education, physical and occupational therapy and other therapies) and pharmacologic therapy including non- opoid oral and topical (i.e., applied to skin) analgesics. For patients who are unresponsive to this regimen, the use of non-steroidal anti- inflammatory drugs (NSAIDs) is considered appropriate. Corticosteroid injection is recommended for patients with knee OA, particularly when signs of local inflammation with joint effusion are present [8, 9, and 10].

Conventional pharmacological approaches to symptom management in OA involve nonsteroidal anti-inflammatory drugs, selective cycloxygenase-2 inhibitors, and intra-articular injection of hyaluronan or corticosteroids. However, there are accumulating data showing that any of these pharmaceutical drugs frequently produce insufficient benefit, with an associated risk of untoward side effects. Glucosamine and chondroitin sulfate are cartilage extracellular matrix components that have been widely used as alternative medicines or nutraceuticals for the management of OA and have been the subject of a huge number of clinical studies for this purpose. Some clinical trials thus far reported support the demonstrated favorable effects of glucosamine and chondroitin sulfate alone or in combination in relieving OA pain. However, none of a larger number of randomized clinical trials gave such positive results, suggesting an ambiguity of the benefit of these two nutraceuticals in OA [11, 12]. Therefore, researchers are now trying to find ways of enhancing the chrondoprotective effects by using different agents along with these nutraceuticals.

In one of the trials positive outcomes were found when treating cultured chrondocytes with Glucosamine, chrondoitin sulphate, & Vitamin C [12]. Also, as per studies Ascorbic acid stimulates collagen synthesis and modestly stimulates synthesis of aggrecan (a proteoglycan present in articular cartilage). [13] As per the Framingham Osteoarthritis Cohort Study, a moderate intake of vitamin C (120-200 mg/day) resulted in a three-fold lower risk of OA progression. The association was strong and highly significant, and was consistent between sexes, among non-supplement users, and among individuals with different severities of OA. The higher vitamin C intake also reduced the likelihood of development of knee pain. [14]

Therefore, considering the above studies, in this study, an attempt shall be made to find out the efficacy of Glucosamine, chrondoitin sulfate, vitamin C in reduction of pain and improve the quality of life in patients with OA.

Research Protocol

<table>
<tr><td>11.</td><td colspan="3">Rationale of the study:
Osteoarthritis of is a fairly common cause of disability in older people. The risk of disability is so great that it is now a costly burden to society and loss of productivity. So, the objectives in managing the patient with OA are to reduce/eliminate pain & stiffness, maintain/improve mobility, optimize function & hence minimize disability.
Glucosamine hydrochloride and Chrondoitin sulfate has been used in OA for a long time now. Therefore, the rationale for this study would be to see improved treatment outcomes combining Gucosamine hydrochloride, Chrondoitin sulfate & Vitamin C</td></tr>
<tr><td>12.</td><td>Research Question</td><td colspan="2">Does Glucosamine, Chrondoitin sulfate & Vitamin C reduce pain in patients with OA?</td></tr>
<tr><td>13.</td><td colspan="3">Aims and objectives:
General objectives:
• To observe and differentiate the effect of Glucosamine hydrochloride, Chrondoitin sulfate & Vitamin C combination in the management of Osteoarthritis (OA) in comparison to treatment with Chrondoitin sulfate & glucosamine hydrochloride alone.

Specific objectives:
1. Effect of Vitamin C on Chondroitin sulfate and Glucosamine hydrochloride treatment compared to placebo group.
2. Effect of glucosamine and chrondoitin sulfate treatment in comparison to placebo group
3. Comparison between the 3 groups</td></tr>
<tr><td rowspan="2">14.</td><td rowspan="2">Materials and Methods</td><td>a. Main outcome variables to be studied</td><td>• VAS.
• 50 feet walking time in second.
• Tenderness index.</td></tr>
<tr><td>b. Confounding variables</td><td>• Age of the patients.
• Sex of the patients.
• Occupational status.
• Socioeconomic condition.</td></tr>
</table>

Research Protocol

		c. Study population	Patients attending in the department of Physical Medicine, Dhaka Medical College Hospital, who are suffering from Knee OA, are the study population.
		d. Sample size	60 patients with OA knee who fulfill the selection criteria will be taken as sample. They will be divided into three group (Group-A, Group-B and Group-C).Each group will consist of 20 patients.
		e. Screening method	Selection criteria (Both inclusion and exclusion criteria)
		f. Sampling method and groups	Simple random sampling. In **Group-A,** GCC (Glucosamine hydrochloride, Chrondoitin sulfate & Vitamin C) treatment will be given (normal established doses) along with Paracetamol 500 mg thrice daily In **Group-B,** GC (Glucosamine hydrochloride & Chrondoitin sulfate) treatment will be given (normal established doses) along with Paracetamol 500 mg thrice daily **Group-C,** Paracetamol 500 mg thrice daily & ADL instructions will be applied

		g. Inclusion and exclusion criteria	**Inclusion criteria:** • Pain in anyone of knee joint. • Duration of pain >3 months. • Age between 40-70 years. • Morning stiffness < 30 minutes. • Crepitus on active movement. • Bony tenderness. • ESR < 40 mm in 1st hour. • Radiological evidence of OA knee like marginal osteophytes, subchondral sclerosis, cyst, joint space narrowing and osteochondral loose bodies. **Exclusion criteria:** • History of trauma / fall/ sports injury of knee joint. • Genu varus / genu valgus deformity. • History of knee surgery. • Inflammatory arthritis like RA, Spondyloarthropathy. • Infectious disease like Tuberculosis. • Crystal associated artrhropathy like Gout, Pseudogout. • Skin infection over knee joint. • Uncontrolled DM. • surgical treatment of knee joint(s) undergone or its necessity; routine use of health food or medicine containing hyaluronic acid, glucosamine and/or chondroitin sulfate and expected to be continued during the study period; • treatment with bisphosphonates,

			hormones or other medicines that may affect the serum or urine concentrations of biomarkers of bone or cartilage metabolism; • intra-articular hyaluronic acid within 2 weeks or corticosteroids within 3 months before inclusion; • need to undergo such topical or systemic pharmacological treatments during the study period; occasional taking of hard exercise; • a history of osseous or articular diseases other than OA within the past 3 months; • treatment with warfarin, undergoing or needed to undergo during the study period; bronchial asthma or potential for developing allergy to the test supplement; • pregnant women; • nursing mothers or women of childbearing potential; participation
		h. Operational definition	**Osteoarthritis**: Osteoarthritis (OA) is defined as a non-inflammatory disease causing metabolic, structural, biochemical changes in articular cartilage and affecting subchondral bone, joint capsule, synovial membrane and muscles around joint. Consequently it causes pain, limitation of joint movement, disability and a decrease in muscle strength.

Research Protocol

		i. Flow chart showing the sequence of tasks	Annex A
		j. Procedure of preparing and organizing materials	Using data sheet(Annex B)
		k. Nature of controls	Not applicable
		l. Randomization and blinding	Randomization: By lottery. Blinding: Single blinding
		m. Equipment to be used	Data sheet(Annex B)
		n. Procedures of collecting data	Data will be collected from both group-A group-B and group-C in a pre designed data collection sheet from the first visit. Further data will be collected from each patient in every two weeks interval from the first visit for up to 6 weeks. Methods of assessment /estimation: 1. Visual analogue scale. 2. 50 feet walking time in seconds 3. Tenderness index.
		o. Professional assistance from expert	Professor of Physical Medicine will be available for taking necessary measures in critical situation arises if any.
		p. Procedure of data interpretation	Interpretation will be performed by using a computer based statistical program SPSS (statistical package for social sciences) for

			windows. Categorical variables will be expressed as proportions (percentages) and numerical data will be expressed as means (standard deviation) and ranges. Tables and graph will be constructed according to the findings.
		q. Quality assurance strategy	In any critical situation, expert opinion will be taken from professor of Physical Medicine. Data collection sheet will be periodically checked by the supervisor of the study.
		r. Time table	1. Literature search review (2 months) May 2013- June 2013 2. Data collection (3-6 months)-July 2013-December 2013 3. Data analysis(1 month)-January 2013
15.	**Ethical implications**	• Institutional permission to collect data will be obtained before conducting the study. No identifiable patient data will be collected and all patients will be coded by serial number. • All patients parties will be explained their conditions in details • Informed written consent will be obtained from the patients or parties. • The study will not interfere with patient management or deal with moral social issue.	
16.	**Total Budget**	Not applicable	
17.	**Source of funding**	Not applicable	
18.	**Facilities available at**	Dhaka Medical College Hospital is a tertiary care hospital	

	place of study	with complete PMR unit with almost all modalities of physical therapy including TENS and all necessary pathological and imaging facilities.
19.	**Other facilities needed**	Not applicable**.**
20.	**Dissemination and use of the finding**	Through seminar in the institution and sending the research report to the index journal for publication.
21.	**References**	1. Peyron JG, Altman RD. The epidemiology of Osteoarthritis. In: Moskowitz RW, Howell D S, Goldberg V M, Mankin H J editor(s), Osteoarthritis: Diagnosis and Management. Philadelphia: WB Saunders, 1992: 15-38. 2. Osiri M, Welch V, Brosseau L, Shea B, McGowan J , Tugwell P, Wells GA.Transcutaneous electrical nerve stimulation for Knee Osteoarthritis (Review). The Cochrane Collaboration. Published by John Wiley & Sons Ltd, 2008; 1:2-8. 3. Fife R S Osteoarthritis: A. Epidemiology, pathology and pathogenesis. In: Klippel J editor(s). Primer on the rheumatic diseases.11th Edition. Atlanta: Arthritis Foundation, 1997:216-8. 4. Solomon L. Clinical features of osteoarthritis. In: Kelly WN, Harris ED Jr, Ruddy S, Sledge CB editor(s). Textbook of Rheumatology. 5th Edition. Vol. 2, Philadelphia: WB Saunders, 1997:1383–93. 5. Mankin J, Clinical features of Osteoarthritis. In: Kelly W N Harris E D jr. Ruddy S Sledge C B editor(s). Textbook of Rheumatology. 7th Edition. Vol.2, Philadelphia: W B Saunders, 2005: 1409-1417. 6. Fargas-Babjak A, Rooney P, Gerecz E. Randomized trial of Codetron for pain control in Osteoarthritis of the hip/knee. The clinical Journal of pain 1989;5(2):137-41. 7.Hochberg MC, Altman RD, Brandt KD, Clark BM, Dieppe PA, Griffin MR, Moskowitz RW, Schnitzer TJ: Guidelines for the

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22.	Any other relevant information	Not applicable

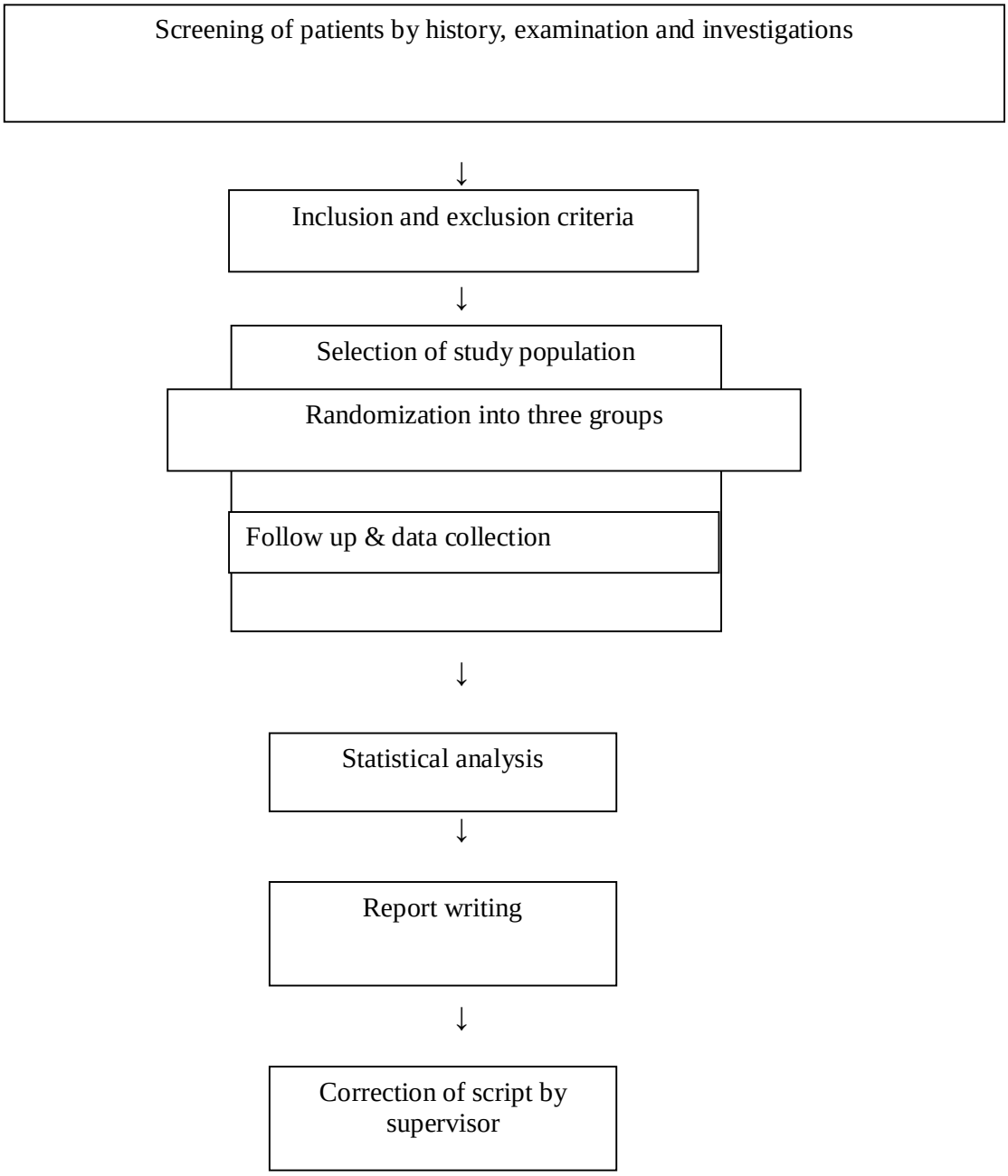
Research Protocol

<table>
<tr><td>23.</td><td colspan="2">Signature of the Chief Investigator:
  </td></tr>
<tr><td></td><td colspan="2">Dr. Repon Kumar Saha, PhD
Assistant Professor
Department of Pharmacy
East West University
Dhaka, Bangladesh</td></tr>
<tr><td>24.</td><td colspan="2">Name and signature of other investigators:</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td></td><td>Ekram Ahmed Chowdhury
Department of Pharmacy
East West University
Dhaka, Bangladesh</td><td>Sadia Tanzin
Department of Pharmacy
East West University
Dhaka, Bangladesh</td></tr>
</table>

Research Protocol

Annex –A

Flow chart showing the sequence of tasks



Annex-B

Department of Physical Medicine & Rehabilitation,

Dhaka Medical College Hospital Dhaka.

Data Sheet

Title: "Role of Glucosamine sulphate, Chrondoitin sulphate & Vitamin C in enhancing chrondoprotective effects and management of pain in OA."

Particulars of the patient:

SL. No.:	Reg. No.:	Date:
Name:	Age:	Sex: Male/Female
Occupation:	Marital status: Married/Unmarried	Socio-economic condition Poor/Middle/Rich
Height:	Weight:	Religion:
Mailing address with Mobile:		Group:

Presenting Complaints:

(A) Knee pain: Right /Left.

Duration: Year ….Month ….Day……

(B) Analysis of Pain:

(a) Onset – Sudden/Gradual/After Trauma/Others

(b) Site of pain – Localized in Knee/ Knee & Other Joints.

(c) Time of occurrence – Morning/ Evening/Night.

(d) Duration of Pain – Constant/Intermittent

(e) Character of Pain – Sharp/Dull/Stabbing/Burring.

(f)Radiation of Pain– No/Yes, (Upwards/Downwards/Both)

(g) Severity of Pain – Mild/Moderate/Severe/Excruciating.

(h) Aggravating factors –stair climbing/walking on uneven surface//prolong standing/ rest.

(i) Reliving factors – Rest/activity/heat/cold.

(j) Nature of Pain – Inflammatory/Degenerative.

Research Protocol

(C) Morning Stiffness: Yes/No (Duration: ……..)

(D) Swelling of Joints: Yes/No (Mild/Moderate/Huge)

(E) Disability: Problem in walking/Squatting/bed ridden.

Treatment History: NSAID/Thermotherapy/Exercise/ADL/Intra-articular steroid injection.

Personal History: Smoker – Yes/No (Sticks…….. per day)

Betel leaf – Yes/No (…… per day)

Drug abuse – Yes/No (Type……..)

Alcohol intake–Yes/No (Regular/Irregular,amount--per day)

Family History:

Associated condition: PUD/DM/HTN/Bronchial Asthma/Heart ,Liver & Kidney Disease.

Past History: Trauma/Aspiration/Surgical intervention/Other Rheum. Disease

General Examination:

Appearance -	Body Built-
Co-operation -	Pulse–
Anemia -	B.P -
Jaundice -	Weight-
Temp. -	Height-
	BMI-

Local Examination:

(A) Examination of the Knees:

(a) Contour – Normal / Swelling

(b) Local Swelling – Absent/ Present

(c) Local Tenderness – Absent / Present

(d) Local Temperature – Normal /Raised.

(e) Eliciting fluctuation – Absent /Present

(f) Leg Length discrepancy - Yes/No –Rt./Lt……cm

(g) Deformity – G. varus/ G. valgus/ Recurvatum/ Normal

(h) Skin changes –

(B) Test for Patella

(a) Position – Normal/Shifted – high/Low

(b) Shape –Normal/Broadening

(c) Mobility – Normal/Painful

(d) Tenderness – Present/Absent

(e) Patellar Tap – Present / Absent

(C) Movement of Knee

(a) Range of motion (ROM) ($0\text{-}135^0$)

(b) Measurement from heel to buttock distance with leg fully flexed ($1cm=1.5^0$)

(D) Test of Ligament

(a) Anterior drawer test

(b) Posterior drawer test

(c) Valgus stress test

(d) Varus stress test

(e) Mc Murray maneuver for menisci

(E) Examination for Gait

(a) Gait Pattern

(b) 50 feet walking time in seconds

(F) Neurological Examination:

(a) Power of the muscles: Upper Limb/Lower Limb.

(b) Wasting of the muscles: UL/LL (Measurement……. cm)

(c) Sensory Change: Yes/No (Site……..)

(d) Knee Jerks: (……)/Ankle Jerks: (……..)

(G) Examination of Hip & low back region:

(a) Straight leg raising test: Positive (Rt./Lt.)/Negative

(b) ROM of hip joints: Normal/ Restricted

(c) Lasague test: Positive/Negative

Systemic Examination:

(A) Locomotor System :

(B) Nervous System :

(C) Cardiovascular System :

(D) Gastrointestinal System :

(E) Respiratory System :

(F) Others System :

Clinical Diagnosis:

(A) Right: Patellofemoral /Tibiofemoral / (Medial/Lateral) OA

(B) Left: Patellofemoral/Tibiofemoral/ (Medial/Lateral) OA

Investigations:

(A) Routine & Serological test

TC:	DC:
ESR:	Hb%:
RBS:	S. Creatinine:
CRP with titre	SGPT:
RA test:	ECG:

Urine for R/M/E:

If needed: S. Uric Acid:

MT test:

Synovial fluid analysis:

(B) Radiological investigation:

(a) X-ray of the knee joint both /Skyline view.

(b) X-ray of the lumbosacral spine both view (If needed).

Confirm Diagnosis:

Research Protocol

Treatment:

Group A: GCC treatment will be given (normal established doses) along with Paracetamol 500 mg Tablet thrice daily & ADL instructions for upto 6 weeks

Group B: GC treatment will be given (normal established doses) along with Paracetamol 500 mg Tablet thrice daily & ADL instructions for upto 6 weeks

Group C: Paracetamol 500 mg Tablet & ADL instructions for the same duration.

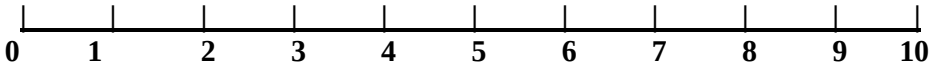
Code No:-

Assessment & follow up table

Research Protocol

Parameters	Pre treatment W0	Post treatment		
		W2	W4	W6
(1) Visual analogue scale VAS (1-10)				
(2) 50 feet walking time, seconds				
(3)Tenderness index				

1. Visual analogue scale (VAS):



In VAS patient, he/she describes the visual impression of his/her pain, Zero means no pain at all and 10 means extreme pain as it is not tolerable by the patient. On the other hand 5 mean medium pain and can be tolerated by the patient. Thus they point out the actual point of pain in the scale and it was documented in the data sheet.

2. 50 feet walking time in seconds: How many seconds a patient has to spend to cross 50 feet distance.

3.Tenderness Index:

0 = No pain

Research Protocol

1 = Describes pain

2 = Patient winches

3 = Patient winches and withdraw the affected part

4 = The patient will not allow the joint to be touched

Research Protocol

INFORM CONSENT FORM FOR PATIENTS

(English Version)

Title:

Role of Chondroitin sulfate, Glucosamine hydrochloride and Vitamin C in management of pain in OA.

Principal investigator: Repon Kumer Saha, PhD.

I am informed by Dr. Repon Kumer Saha about a research work which is being conducted in Dhaka Medical College Hospital on Study of Role of Chondroitin sulfate, Glucosamine hydrochloride and Vitamin C in management of pain in OA**.** I could understand that the outcome of this research work in near future will bring beneficial result on the outcome of this type of disease.

I am hereby, knowing everything and being in good health and mind give my consent to take part in this research work.

Signature of participant or his/her guardian & date

Signature of researcher & date

Name:
Address: