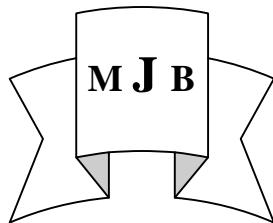


Sex-Pheromone System and Plasmid Transfer in *Enterococcus faecalis*

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Review Article



Abstract

Pheromone-inducible plasmid transfer is an important mechanism for dissemination of antibiotic resistance and virulence factors in these organisms. Plasmid-free strains of *Enterococcus faecalis* secrete at least half a dozen peptide sex pheromone-probably many more-that induce a mating response by potential donor strains carrying members of specific families of conjugative plasmids. The response is associated with synthesis of surface proteins that facilitates formation of donor-recipient mating aggregates. When a recipient acquires a given plasmid , the corresponding pheromone becomes shutdown or masked. Although the structure of at least five of these peptides has been determined.

I-Gene Transfer Among Enterococci:

Transfer of genes among genera of bacteria occurring in two mechanisms; one mechanism, transfer of DNA within bacterial cells by transposons. The transposons are pieces of DNA that move readily from one site on the bacterial chromosome to another site, or they transfer to plasmid. The transposons are not capable of independent replication, they replicate as part of recipient DNA. They can code for drug resistance enzymes, toxins production or variety of metabolic activities. Another mechanism ,transfer of genetic information from one bacterium to another. These transfer can occur by three methods: conjugation, transduction and transformation [1]. Enterococci posses ability to horizontal exchange of genetic materials among themselves and with other gram-positive bacterial genera [2]. The exchange of genetic elements of *Enterococcus*

feacalis by conjugation is studied during twentieth century in deeply. The first convincing evidence for the existence of plasmid-mediated gene transfer in this organism was obtained by Tomura *et al.*, 1973[3] and by Jacob and Hobbs ,1974[4]. At least three conjugative systems have been reported in *Enterococcus faecalis*, (i)The conjugative system occurs in wide range of related bacterial genera(broad-host range plasmids). The plasmids can transfer among enteroccoci species and with other genera such as many species of streptococci, *Staphylococcus aureus*, *Lactobacillus* species, *Bacillus subtilis*, *Listeria monocytogenes* and others. The transfer of this type is largely dependent on forced cell contact on membrane filter and is very inefficient in broth . The transfer of plasmid is lower than(10^{-4} - 10^{-5} per donor cell) gene transfer by pheromone induction[5]. (ii)The transfer of plasmids by transposon mechanism

that occurs at low frequency on filter, and many explain the spread of R-gene to many different species . As opposed to ordinary transposons , which can jump within cell from one DNA location to another, conjugative transposons also encode the ability to bring about conjugation between different bacterial cells[6, 7 ,8].

(iii)Third type involves the plasmid transfer occurs only between *E.feacalis* strains(narrow-host range plasmids). The plasmid-encoded antibiotic resistance and virulence factors can be transfer at high frequency by conjugation (reaching 10^{-1} per donor cell). The transfer conjugative plasmids from donor to recipient cells are inducing by signaling peptide, sex pheromone. Therefore are believed to contribute to horizontal dissemination of antibiotic resistance and virulence factors [9,10].

Sex pheromone system is one of many chemical communication in bacteria especially in gram positive bacteria. In gram positive bacteria several important processes are controlled by cell to cell communication which are mediated by extracellular signal molecules. Most of these molecules are peptides or modified peptides. Signal processing ,in most cases ,involve either transduction across the cytoplasmic membrane or import of the signal and subsequent interaction with intracellular effectors[11].

II-Historical Background of Sex-Pheromone :

In *E. feacalis* , the exchange of plasmids among themselves and other genera is unique involved the production of sex pheromone , while other species of enterococci have the capacity to acquire and exchange is not pheromone induced [12].

E. feacalis contains more than 47 plasmids . At present ,the main know types of plasmids;(i) small, cryptic plasmids, (ii)large, conjugative plasmids and (iii) sexual, pheromone-responding plasmids , up to now , approximately, 20 different pheromone-responding plasmids[3].

Historical , the sex pheromone system of *E.feacalis* was discovered and first described in 1978 by Dunny group[13]. The work by Dunny group reveals the existence of mechanism of regulation of the genes involved in conjugal transfer, mediated by peptide sex pheromone [14]. The earliest investigations of pheromone-inducible transfer of genes during observation of clumping induction assay in mating mixtures or in donor cell cultures incubated with recipient cultures filtration provided the first indication of existence of pheromone , therefore Dunny ,use the term clumping-inducing agent (CIA) to describe these substances [13, 15].

The pheromone is extracellular small peptide (oligopeptide) consist of seven to eight amino acids in length ,and has low molecular weight (less than 1000). The peptide is chromosomally encoded in recipient cells (plasmid-free cells)[15].

E.feacalis cells excrete multiple pheromones. Up to now, five or more different sex pheromones are identified and five specific peptide inhibitors . The peptide inhibitors encoded by responder plasmid in donor cells[16]. Each pheromone is specific for induction of a single plasmid or family of closely related plasmids. They are named according to their respective sex-pheromone-plasmid , e.g: cAD1 (c standing for clumping), iAD1(I standing for inhibitor), and induced plasmid, pAD1 (p standing for plasmid) [17] .

The types of sex pheromones in *E.faecalis* and pheromone-responding

plasmids have been described in table-1.

Table 1: Enterococcal sex-pheromones and the pheromone-responding plasmids described in *Enterococcus faecalis*[18].

Sex-Pheromone	Pheromone inhibitor	Original strain	Pheromone-responding plasmid	phenotype (the plasmid encodes for)
cAD1	iAD1	DS16	pDA1	Hly/Bac
		DS5	pAM γ 1	Hly/Bac
		JH1	pJH2	Hly/Bac
		HH2	pBEM10	Pn ^r ,Gm ^r ,Km ^r ,Tm ^r
		pX98	Hly/Bac
cPD1	iPD1	39-5	pPD1	Bac
		S-48	pMB2	Bac
cCF10	iCF10	SF-7	pCF10	Tc ^r (Tn925)
		T1-4	pMB1.1	Bac
cOB1	iOB1	5952	pOB1	Hly/Bac
		pYL1	Hly/Bac
cAM373	iAM373	RC73	pAM373	cryptic
Derivates	cAM γ 2	DS5	pAM γ 2	Bac
	cAM γ 3	DS5	pAM γ 3	cryptic
	cAM323	HH22	pAM323	Em ^r
	cAM324	HH22	pAM324	cryptic

III-Biosynthesis of sex pheromone in *E.faecalis*:

Many theories are suggested about synthesis of bacterial sex-pheromones in *E. feacalis* [12, 19]. The best-studied sex pheromone is cCF10 as follow:

In plasmid-free cell, the *Ccf-A* gene encodes a secreted precursor of cCF10 (cCF10 p). The precursor is lipoprotein. The cCF10 within the carboxy-terminal end of signal sequence of lipoprotein CcfA.

The AS lipoprotein is secreted across cytoplasmic membrane and anchored to cell wall , signal peptidase I, cleaves

before the cystein residue contained within the conserved lipobox processing site , liberating the signal peptides.

Further proteolytic processing most likely occurs in the cell wall by Eep (enhanced expression of pheromone), which cleaves at the amino-terminal end of cCF10 peptide sequence , and release pro-cCF10.

Final processing may be carried out by exo-peptidase which cleaves off the remaining three c-terminal residue, resulting in mature cCF10, and release the pheromone into the medium, but a

substantial portion remain associated with cell wall of organism [16, 20]. Purification and chemical analysis of several sex pheromones have been

accomplished by Suzuki *et al*(21); Mori *et al.*[22], and Nakayama *et al.* [23](see table-2)

Table 2 Amino acid sequences of sex-pheromones and their inhibitor that are produced by *Enterococcus faecalis* [24]

Pheromones and their inhibitors		Amino acid sequences
pheromones	cCF10	NH ₂ -Leu-Val-Thr-Leu-Val-Phe-Val-COOH
	cPD1	NH ₂ -Phe-Lue-Val-Met-Phe-Leu-Ser-Gly-COOH
	cAD1	NH ₂ -Leu-Phe-Ser-Leu-Val-Leu-Ala-Gly-COOH
	cAM373	NH ₂ -Ala-Ile-Phe-Ile-Leu-Ala-Ser-COOH
	cOB1	NH ₂ -Val-Ala-Val-Leu-Val-Leu-Gly-Ala-COOH
inhibitors	iCF10	NH ₂ -Ala-Ile-Thr-Leu-Ile-Phe-Ile-COOH
	iPD1	NH ₂ -Ala-Ile-Leu-Thr-Leu-Val-Ser-COOH
	iAD1	NH ₂ -Leu-Phe-Val-Val- Thr-Leu-Val-Gly-COOH
	iAM373	NH ₂ -Ser-Ile-Phe-Thr-Leu-Val-Ala-COOH
	iOB1	NH ₂ -Ser-Leu-Thr-Leu-Ile- Ser-Ala-COOH

IV-Mechanism of Plasmid Transfer by Sex Pheromone Induction:

Pheromone-inducible plasmid transfer is a novel form of bacterial conjugation which has several important stages of interaction between the donor cells and recipient cells. A model for the function of the sex pheromone system was first formulated by Ike and Clewell,1992 [25]. In briefly the following mechanism of transfer of plasmid by sex pheromone in *Enterococcus faecalis*(as illustrated in figure-1).

The initial interaction: The plasmid-free cells produce sex pheromone peptide into medium. This peptide act as chemical signal which transmitted from the recipient cells to donor cells, and the

pheromone interact with receptor on surface of donor cells via specific binding by plasmid-encoded lipoproteins(*Prg-Z* gene).

The subsequent import of the pheromone into cytoplasm of responder cell via chromosomal oligopeptide permease (Opp). The Opp act as membrane translocator which composed with two transmembrane pore-forming subunits (Opp-B, Opp-C) and two cytoplasmic ATPase(Opp-D ,Opp-F). The later subunits are used with the energy, it require to ATP-binding cassette (ABC) for active transport of import peptide through Opp.

The imported pheromone into cytoplasm is interact with intracellular effectors

molecules, *Prg-X* gene , is negative regulation control. This binding of pheromone with *Prg-X* gene abolish it negative control functions, these include synthesis of two surface proteins: aggregation substanc(AS) and exclusion substance(ES) on surface of donor cells . The plasmid encoded protein (AS) which reacts with chromosomal encoded receptor ,binding substance(BS) , on surface of recipient cell. The binding between AS and BS is require divalent cations(Mg^{+2} , Mn^{+2} , Ca^{+2} , Co^{+2}) and phosphate ions. This binding which responsible for cell-cell contact and formation of mating channel, then single stranded of plasmid is transferred to the recipient cell.

The shutting off ; after the plasmid has established itself in the recipient cell

results in shutting off the activity of the pheromone by two functions encoded on the plasmid; one involves a reduction of the pheromone production, and other by production of specific inhibitor peptide which encoded by *prg-Q* gene. The inhibitors competitive with exogenous pheromone by bind to pheromone receptor.

The surface substances(ES proteins) which induced by pheromone also has important role in the prevention of plasmid transfer between aggregated donor cells. The *Prg-Y* proteins are expressed to prevent self induction by endogenous pheromone which produced from donor cell, through degrades or inhibits the activity of the pheromone in cell wall of donor cell. [9,16,26,27].

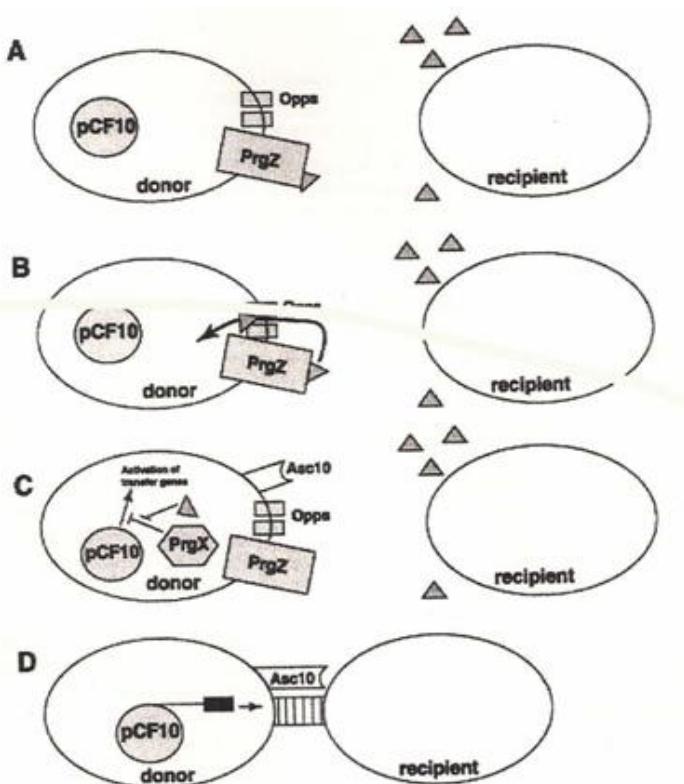


Figure 1: Summary of major steps in pheromone-induced conjugation[26].

In Iraq, AL-Barzangi 2001 , she found fifty five percent of culture filtrate of *Enterococcus faecalis* were able to produce clumping-inducing agent(sex-pheromone)which facilitate plasmid DNA transfer by conjugation and rise frequency of conjugant cells[28]. The results of other study in our country which performed by AL-Khafaji, 2005 showed the possibility of isolation and purification of sex-pheromone,cCF10, by using ion-exchange chromatography. This purified sex pheromone from culture filtrate was active in an induction of transferring resistance trait to tetracycline antibiotic drug among *Enterococcus faecalis* isolates. The frequency of conjugation was increased in high level , 1.6×10^{-1} . The effect of cCF10 sex pheromone on plasmid transfer can be controlled by using protein synthesis-inhibition antibiotic agents such as chloramphenicol [29].

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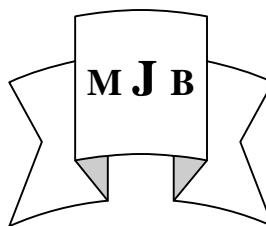
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Glucosamine and Chondroitin Sulfate in Knee Osteoarthritis

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Abstract

Osteoarthritis common disease of knee joint , due to aging specially after age of 40 , it disorder of synovial joint .the glucosamine and chondroitin provide much of the resistance to compression and its used now for the treatment .the aim of this study to show the effect of this dietary supplement in knee osteoarthritis ,between 2006 &2008 in merjan teaching hospital 120 patients was selected according to ACR criteria & divided in two groups 60 for each ,they examined and follow-up regularly for six months ,group [1] given the dietary supplement ,the other given NSIAD [meloxicam],the knee joints of both groups examined at regular interval to show the response according to different parameter ,the functional score show improvement 31 while group [2] show 26 and the visual analogue score was more improved in group [1] than in group [2] and the radiological score show no difference

In this study the dietary supplement show rapid improvement with maximum at 4 months while other group show improvement at 2 months with no more improvement , the side effect in group [1] were less in group [2].

The glucosamine and chondroitin should be uswd as alternative drugs tn NSAIDs because less side effect and better response in different functional score.

عقار كلوكوزامين والكوندروتين في التهاب المفصل الضموري للركبة

الخلاصة

التهاب المفصل الضموري مرض شائع يصيب الركبتين نتيجة تقدم العمر وخصوصا بعد العقد الرابع من العمر, وهو مرض يصيب المفاصل الزلالية .الكلوكوزامين والكوندروتين تزود القسم الأكبر من المقاومة للانضغاط وهو يستخدم حاليا في علاج هذه الحالة .إن الهدف من هذه الدراسة هو لدراسة تأثير هذه المكمالت الغذائية في علاج التهاب المفصل الضموري للركبة وقد أجريت بين عامي 2006 و 2008 في مستشفى مرجان التعليمي في بابل ,120 مريضا تم اختيارهم حسب المعايير الخاصة لكليمة المفاصل الأمريكية وتم فصلهم إلى مجموعتين 60 مريضا لكل مجموعة ،المجموعة الأولى عولجت بعقار الكلوكوزامين والكوندروتين والأخرى عولجت بعقار المليكسوكام .وتم فحص مفاصل الركبتين لكلا المجموعتين في فترات محددة لغرض قياس الاستجابة اعتنادا على قياسات مختلفة وكانت النتيجة الوظيفية ومقدار النتيجة البصرية للألم للمجموعة الأولى أفضل من المجموعة الثانية ولكن النتيجة الإشعاعية لم يتغير طول فترة الدراسة

في هذه الدراسة دلت على ان المكمالت الغذائية أدت إلى تحسن أسرع يصل إلى الحد الأعلى في فترة 4 أشهر لكن العلاج الآخر تبدأ الاستجابة في شهرين مع عدم تغير في معدل الاستجابة مع استمرار فترة الدراسة كما إن المضاعفات الدوائية أقل في المجموعة الأولى إن عقار الكلوكوزامين والكوندروتين يفضل أن يكون بديلا للأدوية لا ستيرويدية كونها أقل من ناحية المضاعفات الدوائية وتظهر استجابة أسرع في النتيجة الوظيفية والبصرية

Introduction

Osteoarthritis is most common form of joint disease , its disease of aging

, 90% of all people have Radiographic features of Osteoarthritis in weight bearing joints by age of 40, symptomatic

disease also increase with age. the most important clinical aspect in this disease is pain and impairment of movement. This arthropathy is characterized by degeneration of cartilage and hypertrophy of bone at the articular margins , inflammation usually minimal, Hereditary and mechanical factors may involved in Pathogenesis[1].

Osteoarthritis is a disorder of Synovial Joints . The most frequently affected sites are apophyseal joints of the cervical and lumber spine & knee & hip , unlike inflammatory arthropathies , it does not always affect whole joint . e.g is effect medical tibiofemorel & lat Pattalofemorel compartment of the knee [2]

Chondroitin Sulfate is an important structural component of Cartilage and provides much of its resistance to compression , along with glucosamine , chondroitin Sulfate has become a widely used dietary supplement for treatment of Osteoarthritis[3] .

They are major component of extracellular matrix & important in maintaining the structural integrity of the tissue. Both found in dietary supplements used as an alternative medicine to treat Osteoarthritis & also approved and regulated as a symptomatic slow acting drug for this disease.

The benefit from them are likely the result of a number of effects including its anti inflammatory activity, the stimulation of the synthesis of proteoglycans and Hyaluronic acid, inhibiting the synthesis of proteolytic enzymes [4], nitric oxide and substances that contribute to damage cartilage matrix and cause death of articular chondrocytes[5].

Aims of Studies

The study is aimed to show the effect chondroitin- glucosamine – dietary supplement benefit in the treatment of OA knee patients & compare with other drug to treat patient & improve functionally disability of knee joints in Iraqi patients.

Patients and Methods

Study was done in Hilla in privet clinic. Between 2006-2008 / Babylon Province. Patients were selected according to these criteria :

Patient al either sex more than 50 years & they fulfilling the American collage of Rhenmatology (ACR) criteria of clinical, laboratory and radiographic findings[6], those patients were diagnosed with primary osteoarthritis with lequesns score in the range of (10-18) [7]& those patients can come for regular visit. 150 patient were included in the study ,30 patients were excluded because either they had arthritis due to other cause like Rheumatoid arthritis or have renal or hepatic dysfunction or other serious medical illness (Sever Ischemic heart Disease. Or heart failure.

The first screening visit , Patient medical history was taken & clinical assessment was done . The knee its were examined by local examination & specific parameter for assessing the severity subjectivity as well as objectivity.

All patients were asked to guard the severity of pain based on visual analogue scale (VAS) ranging from no pain at bottom of the scale to unbearable pain at the top of 100 mm colored scale. The other variable in the scale were mild , moderate and sever pain separated by 20 mm. [8] , lequesnes index (Table 1)

Table 1 Lequesne's index (Functional Index for OA of Knee).

Pain or Discomfort	Index [†]
<u>During Nocturnal Bed rest:</u>	
None or insignificant	0
Only on movement or in certain positions	1
With no movement	2
<u>Morning Stiffness Or Regressive Pain After Rising:</u>	
1 minute or less	0
More than 1 but less than 15 minutes	1
15 minutes or more	2
<u>After Standing For 30 Minutes</u>	
<u>While Ambulating:</u>	
None	0
Only after ambulating some distance	1
After initial ambulation and increasing with continued ambulation	2
After initial ambulation, not increasing with continued ambulation	1
<u>While Getting Up From Sitting Without The Help Of Arms</u>	
<u>Maximum Distance Walked (may walk with pain):</u>	
Unlimited	0
More than 1 km, but limited	1
About a km in about 15 min	2
From 500 to 900 m about 15 min	3
From 300 to 500 m	4
From 100 to 300 m	5
Less than 100m	6
With one walking stick or crutch	1
With two walking sticks or crutches	2
<u>Activities Of Daily Living:</u>	
Able to climb up a standard flight of stairs	0 to 2
Able to climb down a standard flight of stairs	0 to 2
Able to squat or bend on the knees	0 to 2
Able to walk on uneven ground	0 to 2

[†] Without Difficulty: 0; With Small Difficulty: 0.5; Moderate: 1; Important Difficulty: 1.5; Unable: 2.

which is functional scoring system was measured accordingly[8].

The objective assessment of the knee joint was done by noting the presence or absence of swelling , deformities , tenderness, warmth, crepitus, joint effusion – and muscle atrophy & the joint mobility was assess by patients. Global assessment done by all these parameter measured by physician & patients, recorded very poor , poor, good, very good[9].

Laboratory assessment such complete blood picture (CBP), Random Blood Sugar (RBS) and electro Cardiogram, and Liver and Renal Function Test for inclusion and exclusion of patient first assessment of patients also done at end of the study .

Knee joints X Ray were done for each patients , for each follow up ((at 2 months interval)) , The wave graded by a scoring system of 1-4 by the radiologist (Table 2)[10].

Table 2. Radiological scoring for Knee osteoarthritis

Radiological scoring	X-ray finding
0	Normal
1	Doubtful narrowing of joint space/possible osteophytes lipping
2	Definite osteophytes/absent or questionable narrowing of joint space
3	Moderate multiple osteophytes, definite narrowing of joint space, some sclerosis, possible joint deformity of bone ends
4	Large osteophytes, marked narrowing of joint space, severe sclerosis, definitive joint deformity of bone ends and subchondral cysts may be present.

120 patient were included , divided into: two groups for period of 6 months follow for each patient with regular visit at 2 months interval between.

The Group (1) 60 patients were give combinations of 500 ghcosamine sulphate& 400 Chondnotion sulphate. 2 tablet per day.

The Other Group (2) 60 patients were give Meloxicam 15 mg / Day.

All Patient to instructed not take any analgesic except paracetomol (only if needed during the study period . The Except no more 1000 mg / day and they asked at each visit how much they have taken of the drug .

Patients were follow up 0- 2 – 4 – 6 – months :

The knee joints were examined on each visit according to above parameter . The Patient compliance was considered good if the patient takes 75% of drug which prescribed . All Patients were advised about knee exercise at home .

Results

This study was design as compared study between [two] groups group one and two both include 60 patients for

each ,the mean age of group [1] was 57 years and group [2] was 58 years.

Sex ratio ,group [1],51 female and 9 male and group [2] 50 female and 10 male table [1].

The functional score was recorded for both groups from the start of the study and at 2 months interval to the end of the study ,in group [1] the improvement was 30-/+ 5 while group [2] was 25-/+5 at the end of the study table [2].

The visual analogue scale for both groups were recorded at the start and 2 months interval to the end of the study ,the improvement from 70-/+6 to 35 in group [1] where the improvement in group [2] was from 72 to 50 table [3]

The radiological score ,there was no difference between both groups during the study period and no difference in progress of osteoarthritis radiological features. table[4].

To compare the use of additive drug [paracetomol] by the numbers of the tablets [more than 10 tablets] per week ,in group one 41 patients had recorded to use the drug while group [2] 38 patients were use the drug. table[5] .

The patient and physician assessment during the study the difference was more

in patients self assessment than physician assessment .,58% of group [1] patients showing improvement at the end of the study while group [2] only 45% show improvement ,the physician assessment show that group [1] 55% of

patients and group [2] only 52% at end of the study ,table[6]
The hematological and biochemical follow up of both groups at the start of the study and end of the study showed in table[7].

Table I : group I & II

Group I	Group II
No = 60 patients	No = 60 patients
Sex F 51[85%] = M 9[15%]	Sex F 50[83%] = M 10[17%]
Mean age = 57 year	Mean age = 58
Duration clinical Symptom = 3-5 year	Duration clinical Symptom = 3-5 year

Table II : Functional scores

Group	0	2months	4months	6 months
G I	14 + 5	18	25 + 5	30 + 5
G II	15	16	18	22 + 5

Table III : Visual analogue score :

Group	0	2months	4months	6 months
G I	70 + 6	60	45	35
G II	72	55	50	48

Table IV Radiological score

Group	0	2months	4months	6months
G I	2.5	2.4	2.3	2.3
G II	2.5	2.4	2.4	2.4

Table V

Group	No of patients	%
I	41 patient	68.1%
II	38 patient	63.5%

Table VI Patient & physician assessment

Assessment	Group 1	Group 2
Patient	58%	42%
Physician	55%	52%

Table VII

Lab study	I		II	
	0	6 month	0	6 month
Hb %	11.5	11	11.7	10.5
WBC	6.3	6.5	5.7	6.5
ESR	35	30	30	30
Blood glucose	100	110	105	103
B urea	40	41	35	45
AST	27	29	26	35
ALT	25	28	27	32

Discussion

Osteoarthritis is a disease of progressive cartilage damage seen mostly after the age of 40s years ,

The pain may arise from synovial inflammation or periosteal nerve stimulation ,previously NSIADs widely used for relieving the pain in osteoarthritis but mostly there is good present of patients that stop drug because adverse effect that related to the therapy and the older people with multiple clinical problem that NSIADs lead to more adverse effect ,due to this fact there was new drugs emergence that would work to reduce patients symptoms ,and they act to regenerate cartilage and act as anti inflammatory with low risk for patients[11] this supported also by study of Alekseeva et al [12]

The glucosamine and chondroitin combination has been sued in all over the world for knee osteoarthritis and many studies work for that, it used as nutritional supplements for knee osteoarthritis[13].

In this study combination of glucosamine and chondroitin has work well as compare with treatment by meloxicam drug ,the pain score was assessed by the visual scale ,this parameter showed progressive improvement over the period of drug therapy with maximum benefit at 4

months point while the other group show rapid improvement at 2 months point but then no more improvement after that point ,when compare with Bourgeois et al [14], 3 months trial of 1200 mg of both combination in single or divided doses its showed that decrease of lequesne index and spontaneous joint pain score versus placebo [$p<0.1$]

The lequesne index that assess the functional score the functional mobility of the joints depending on the daily activity ,it showed mark improvement than the other group at 2-4 months and such result was documented in other studies e.g Muller et al [14] ,Noeak et al[15] and they found that this index to be a sensitive indicator of the improvement, Muller and collogues [16] in short term study [4 weeks] study to compare this drug versus ibuprofen showed that its effective like ibuprofen and better tolerated [$p<0.1$]

The radiological score change in this study show no difference between 2 groups during the this study period ,they not correlated with patients symptoms many patients show functional disability that not correlated with X ray finding . And only 6% show adverse effect while 35% eboburfen qroup show adverse effect mainly gastrointestinal, in our study CS group show 10% show adverse

effect and the other group 21% show adverse mainly gastrointestinal effect . Many study show and support the use of alternative drug to higher risk group patients of NSAID or cyclooxygenase 2 inhibitor for osteoarthritis

We used the combined medication from the same drug manufacture to avoid the difficulties that found with commixture of the medication that found by leeb et al whom performed a met analysis of 7 trials including 372 patients that cited the difference . While Richy et al study [17]that examined both the structural and symptomatic efficacy of the drug via radiological progression of the joint space narrowing ,the patients in this study which include 1975 patients show significant effect on the joint space narrowing but over 3 years period and the tolerance was equal to placebo ,we used the combined medication from the same drug company because in the market many trade manes from different companies to avoid the difficulty that found with mixtures of the medication that found by leeb et al [18]whom preformed a meta analysis of 7 trials including 372 patients that cited the difference

Conclusion

This drug is beneficial when it used in Iraqi patients with no adverse effect like NSAIDs specially gastrointestinal ,it lead to improve functional ability of the patients and decrease the pain but longer study to be performed to see the effect on the joint space narrowing and other studies to be done to see the effect on others joints

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