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RESEARCH ARTICLE

ALENDRONATE SODIUM IN OSTEOARTHRITIS: EFFECTS ON ANABOLIC, CARTILAGE DEGREDATIVE MARKERS AND THE CLINICAL ACTIVITY.

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Abstract

Objective: Osteoarthritis (OA) is a common arthritic disorder and responsible for 2 % of disability in all world. OA affects all joint parts including cartilage, bone and synovium which characterized by degradation of cartilage, subchondral bone turn over and osteophytes formation.

Aim of the study: To evaluate the effect of alendronate sodium (ALN) on disease activity and physical function, evaluate the biochemical parameters that are related to OA patients and study the impact of ALN on bone anabolic and degradative marker, and to determine its effectiveness in slowing progression of disease.

Patients and methods:116 OA patients over 45 years old with Kellgren and Lawrence X-ray grade II and more were enrolled in this study. Base line assessment was done, Kellgren and Lawrence X-ray grading, WOMAC scoring, body mass index and the biochemical parameters with enzyme-linked immunosorbent assay (ELISA) analysis of serum TGF (transforming growth factor) beta 1 and C-terminal cross linked -telopeptide of type II collagen (CTXII. They were instructed to take ALN 10 mg daily. Reassessment was done after 3 months.

Results : Asignificant symptomatic improvement in WOMAC scoring regarding pain and stiffness were observed associated with significant reduction in serum CTXII, TGF beta 1. A no significant reduction in serum calcium with no significant changes in serum Alkaline phosphatase C-reactive protein function and joint space width were also reported.

Conclusion: ALN in patients with OA has clinical efficacy in reducing symptoms especially pain probably through inhibition of TGF beta 1 with no significant structural improvement despite reduction of CTXII , and may help delay and prevent further disease progression probably through inhibition of TGF β1 activity in the subchondral bone.

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Introduction:-

Osteoarthritis (OA)is a slowly progressive degeneration of articular cartilage with subsequently joint space width (JSW) reduction (Blagojevic*et al*., 2010), a common form of arthritic problem (Abramson *et al*., 2006). In spite the fact that OA has been believed to be principally a cartilage defect associated with focal articular cartilage degradation (Felson, 2006). It has been found that a solid and stable aspects of subchondral bone is needed for articular cartilage in load-bearing articular joints, which affects the activity of subchondral bone in OA (Mahjoub *et al*., 2012 ; Suri and Walsh, 2012).the structural changes throughout OA progression including decreased mineralization of the trabecular structure, amplified turnover in the subchondral bone, defects in bone marrow and subchondral plate sclerosis (Karsdal *et al*., 2014). OA is characterized by a repetitive inflammatory aspect of the articular cartilage due to focal defect or erosion of the cartilage and hypertrophic changes of osteoblastic function or osteophytosis (Esser and Bailey, 2011). These defining abnormalities result in a reduction in JSW leading to painful, immobile, and disabling joint (Ringdahl and Pandit, 2011).Meanwhile, when bone resorption is decreased a range of biochemical investigations has demonstrated positive events on cartilage health (Radin and Rose, 1986). OA is a multifactorial disorders (Berenbaum, 2013). risk factor for OA like age, OA is highly age-related because old cartilage expresses changes in TGF (transforming growth factor)-β signaling with decrease protection capacity (Van der, 2014). Other factors like gender (Patriciaet al., 2011), race(Cruz-Almeida et al., 2014), overstress (Voelker, 2011) and sex hormone (Jorge *et al.* , 2009) . OA can be classified as primary (idiopathic) if its cause is not well defined and secondary when there is a certain events causing OA (Brandt *et al*., 1986).

Cytokines such as TNF-alpha and IL-1 increase matrix metalloproteinases (MMP) gene expression and block chondrocyte balancing pathway (Scanzello and Goldring, 2012). IL-4, IL-10, and IL-13 have the ability to reduce synthesis of both TNF-alpha and IL-1beta, and to control MMP activity(Fernandes *et al*., 2002).IL-1 further affectsthe action of certain growth factors, such as transforming growth factor-beta (TGF- β**)** (Alejandro, 2011). TGF β 1 responsible for maintaining homeostasis between articular cartilage and subchondral bone (Gehua and Xu, 2014).TGF-β1 is found in high level in subchondral bone from humans with OA (Gehua*et al*., 2013). High concentrations of TGF-β1 enhance formation of nestin-positive mesenchymal stem cell clusters, which enhances the generation of marrow osteoid islets associated with high levels of angiogenesis(Gehua*et al*., 2013). It has been found that spinal osteophyte formation is associated with alternation in the TGF-β1 gene in Japanese women (Yamada, 2000; Yamada *et al*., 2000) and targeting TGF β1 could be a therapeutic approach to managing OA patients (Gehua*et al*., 2013).

Findings in OA joints include increase size of bone tissue, crepitus, effusions, and decreased range of motion. Tenderness on palpation and pain on passive movement are also common, even though not exclusive to OA **(**Joseph et al., 2009).Primary OA is typically diagnosed according to clinical and radiographic imaging findings (Hunter, 2008). Kellgren and Lawrence (K-L) system is the most often used (Kellgren and Lawrence, 1957).It has been discovered that a combination of both pharmacological and non-pharmacological therapies exert a beneficial role in managing patients with knee osteoarthritis (Alshami, 2014).

Alendronate sodium (ALN) is a nonhormonal therapeutic agent, synthetic analogs of pyrophosphate attach to the hydroxyapatite, one of bone contents. It considered as a member of bisphosphonates. ALN is a 4-amino-1-hydroxy-1-phosphonobutyl, hydroxyphosphinate,bisphosphonic acid monosodium salt trihydrate.ALN is a potent antiresorptive agent poorly absorbed from the gastrointestinal tract, eliminated by active secretion of drug by renal transport system, with very long half-life (1 to 10 years) of bisphosphonates in bone, which related to different levels of bone turnover among species (Lin, 1996).The bisphosphonate inhibitory action on bone resorption is resulted from accumulation of bisphosphonate in osteoclasts after released from bone surfaces during bone resorption. Bisphosphonates inhibit farnesyl pyrophosphate synthase . This prevents the synthesis of isoprenoid lipids by disruption normal function. Isopentenyl diphosphate metabolite trapped.Thus, bisphosphonate disrupt osteoclast function leading to reduction of bone resorption (Michael *et al*., 2011 ; Frank *et al*., 2011). Bisphosphonates, including ALN were reversing the pathophysiological features, leading to decrease bone turn over and significant increment in mineral density of bone, of postmenopausal osteoporosis (Richard *et al*., 2011). Animal model of study, it has been demonstrated that ALN protects chondrocyte from OA events induced by IL-1beta by increasing Collagen II and reduction of MMP-13 within chondrocytes (Wang *et al*., 2011).

ALN is used in osteoporotic postmenopausal lady (Nijs *et al*., 2006 ; Rogers, 2003), corticosteroid-associated osteoporosis (Nijs*et al*., 2006), paget's disease (Reid andHosking., 2011) and osteogenesis imperfecta (Evans et al.,

2003). In spite of the high benefit of ALN, it induces gastric mucosal damages (İşeri *et al*., 2005), esophageal problem (Naniwa *et al*., 2008). Osteonecrosis of the jaw (Khosla *et al*., 2007).

The certain contraindications to ALN are acute inflammations of the gastrointestinal tract (Cryer and Bauer, 2002), hypersensitivity (Naniwa *et al*., 2008), abnormalities of the esophagus (Naniwa *et al*., 2008), inability to stand or sit upright for at least 30 minutes (Zentiva, 2016), hypocalcaemia (Zentiva, 2016), renal impairment (Miguel *et al*., 2013) and osteomalacia (Lenart *et al*., 2008).

A potential benefit of antiresorptive agents, bisphosphonates, results from experimental studies have shown promising results in treatment of OA (Tim, 2003). ALN intake, in symptomatic hip OA, is effective in pain reduction associated with no significant observation obtained in OA pathology after 2 years treatment(Nishii *et al*., 2013). ALNwas associated with less spinal osteophyte and joint space narrowing progression (Neogi *et al*., 2008 ; Siebelt*et al*., 2014).

Animal model of study has suggested that Local elution of ALN acid leads to a dose-dependent increment of bone formation (Bobyn *et al*., 2014) and the subcutaneous ALN injections for rabbits OA resulted in reduction of cartilage degeneration, stopping of bone loss with observed improvement in subchondral bone microarchitecture (Mohan *et al*., 2013). Both in vitro and in vivo studies have found that ALN has the ability to protect chondrocytes by decreasing MMP-13 expression (Hu *et al*., 2009). ALN has the ability to reduce remodeling of subchondral bone leading to important protective effect on articular cartilage (He *et al*., 2012 ; Mohan *et al*., 2013).TGF beta can be defensive as well as harmful for articular cartilage in OA. High levels of active TGF-β1 in subchondral bone leads to initiation of the pathological events of OA (Gehua*et al*., 2013) and a potential role of TGF beta signaling in OA development were observed, with significant correlation between activin receptor-like kinase 1 (ALK1) receptor, (receptor for action of TGF beta) and MMP-13 expression (van der *et al*., 2010). In rat with anterior cruciate ligament transection model, bone resorption markedly elevated, ALN inhibit Subchondral bone remodeling, which play a role in the OA pathogenesis, suggesting that ALN or other bone resorption inhibitors could potentially express DMOAD (Hayami *et al*., 2004). Old cartilage finds to be less protected by TGF-β and express significant changes in TGF-β signaling pathways. during aging, Loss of the protective Smad2/3 pathway can give an explanation for the relationship between aging and OA(van der, 2014). High-dose ALN leads to complete blockage in the local elevation in MMP-13 and TGF β , which could decrease TGF β enhancement by blocking MMP-13 expression in chondrocytes, important in the pathogenesis of OA, so ALN or other bone resorption inhibitors could potentially be used in OA treatment DMOAD (Tadashi *et al*., 2004). ALN treatment and physical activity exercised increased cartilage content and reduce OA progression (Siebelt*et al*., 2014).

Patients and methods:-

116 OA patients over 45 years old with Kellgren and Lawrence X-ray grade 2 and more will enrolled in this study. The male patients were 32 while the female were 84 giving the male to female ratio of 1:2.63. The patients were habitants of the city of Al-Diwanyhia and Baghdad cities and had the Iraq nationality.Laboratory equipment and reagents were of the highest available grades. Base line assessment were done in form of Kellgren and Lawrence X-ray grading (Kellgren and Lawrence, 1957), WOMAC scoring (Falk et al., 2008), body mass index and the biochemical parameters (serum calcium (Ca), alkaline phosphate (ALK), CTXII (ELISA) and TGF beta 1 (ELISA kit)).OA patients were instructed to take 10 mg alendronate sodium tablet orally at the morning, reassessment was done after 3 months.Statistical analysis**:** Data were expressed as mean standard deviation. Comparison was done using pared t-test.

Results:-

Demographic characteristic of the study population:The total number of cases enrolled in the present study was 116, 32 male and 84 female with a male to female ratio of 1:2.63. mean age of patients was 54.68+5.16 years and the age range was 45-65 years, whereas the mean body mass index (BMI) was 30.21+5.91 and it ranged from 21.87 to 37.81 kg/m^2.

There was no significant change in mean Alkaline phosphatase before and after treatment,with no significant reduction in mean serum calcium and CRP as shown in table (1) and figures (1 and 2) through (3).

Table 1:-Biochemical and serological parameters: Comparison of mean serum Alkaline phosphatase, calcium and CRP before and after 3 months treatment with ALN.

	Before(mg/dl)		After(mg/dl)		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**P-value**
Alkaline phosphatase	105.64	25.69	108.08	24.05	0.055
Serum calcium	9.79	0.74	9.67	1.30	0.245
CRP	9.05	7.93	8.28	8.21	0.221

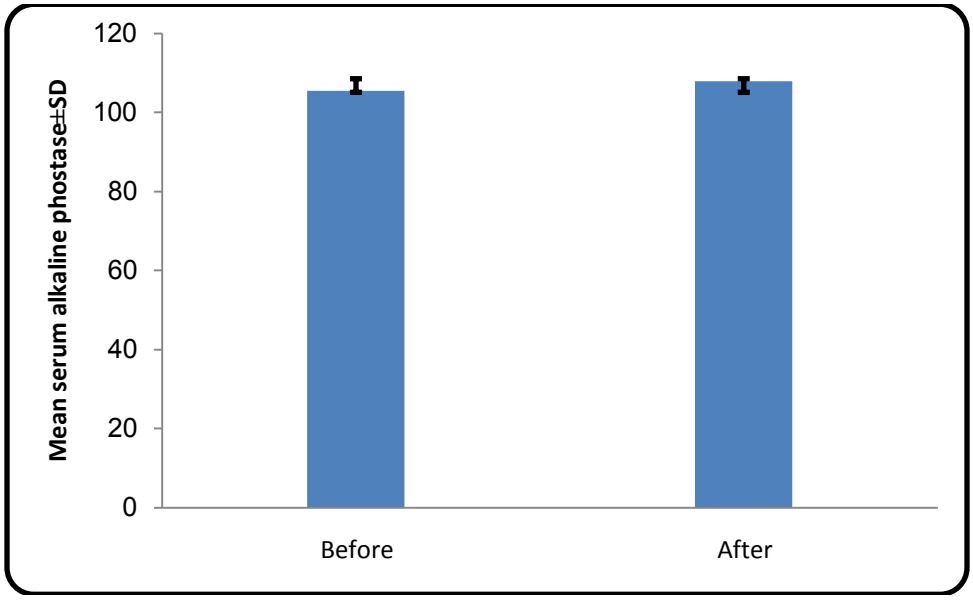

Figure 1:- Mean serum Alkaline phosphatase level before and after 3 months treatment with 10 mg ALN.

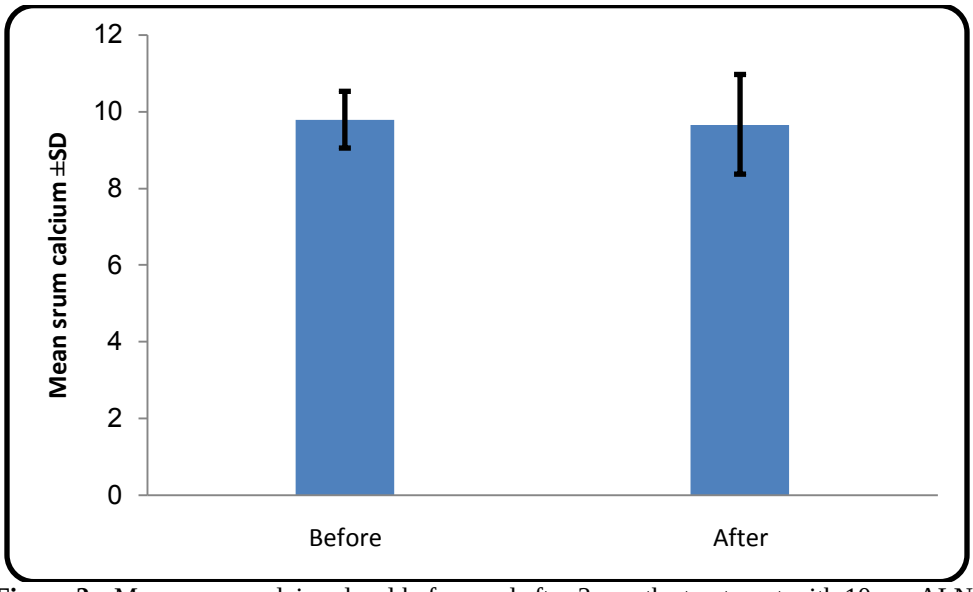

Figure 2:- Mean serum calcium level before and after 3 months treatment with 10 mg ALN.

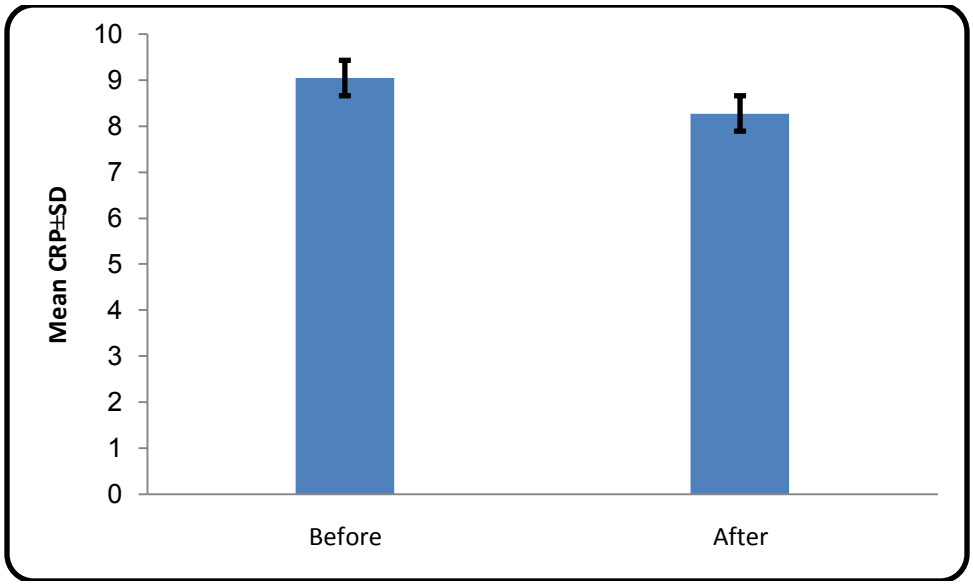

Figure (3): Mean CRP level before and after 3 months treatment with 10 mg ALN.

Table 3:-Changes in mean markers: CTXII and TGF-β levels before and after 3 months treatment with 10 mg ALN.

	Before		**After**		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**P-value**
CTXII	6.93	3.83	4.35	2.66	<0.001
TGF-β1	257.10	219.87	175.12	118.83	<0.001

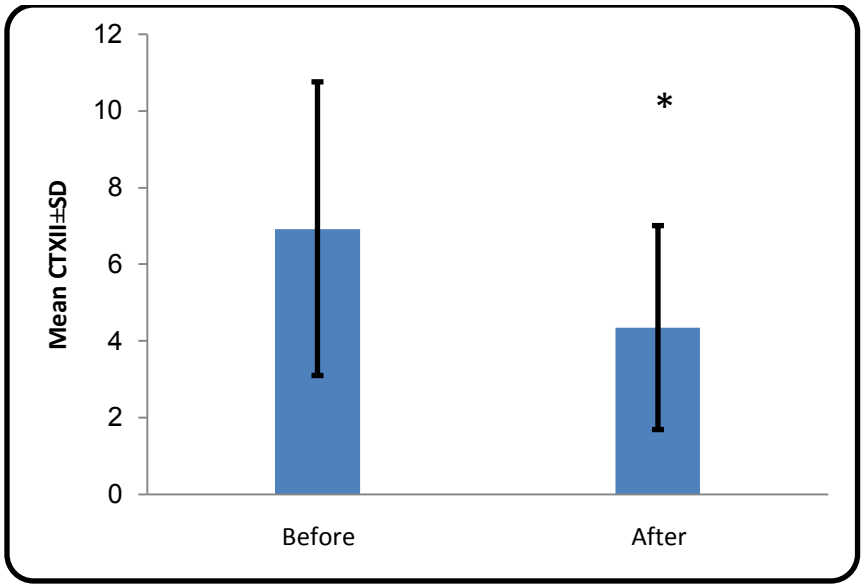

Figure 4:-Mean CTXII level before and after 3 months treatment with 10 mg ALN.

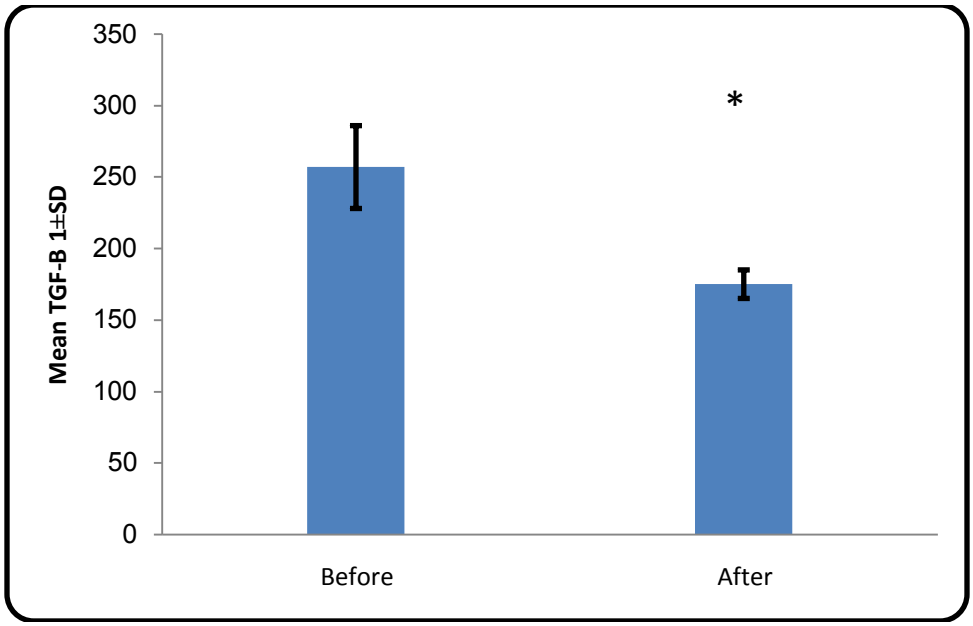

Figure 5: Mean TGF-β1 level before and after 3 months treatment with 10 mg ALN

Table 4:- Clinical parameters including Changes in mean JSW and WOMAC domains before and after 3 months treatment with 10 mg ALN.

	Before		**After**		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**P-value**
JSW(mm)	2.76	0.59	2.80	0.53	0.295
Pain	7.93	1.38	5.41	1.29	<0.001
Function	40.39	6.10	39.54	6.51	0.661

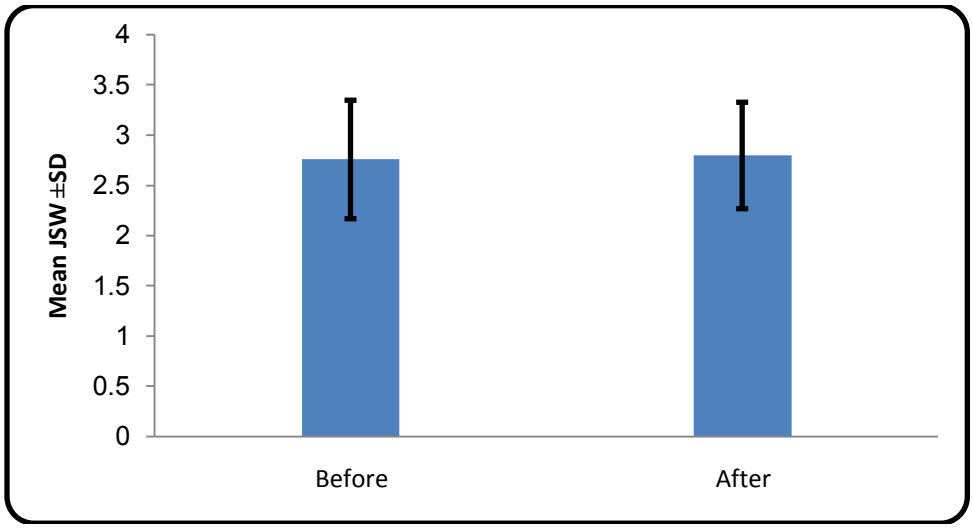

Figure 6:- Mean JSW before and after 3 months treatment with 10 mg ALN.

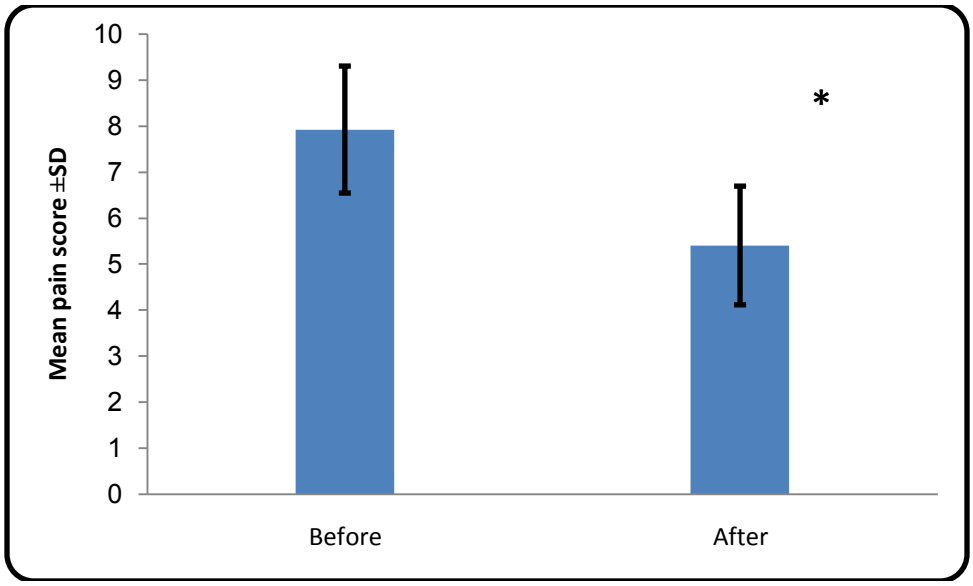

Figure 7:- Mean pain score level before and after 3 months treatment with 10 mg ALN.
* significant

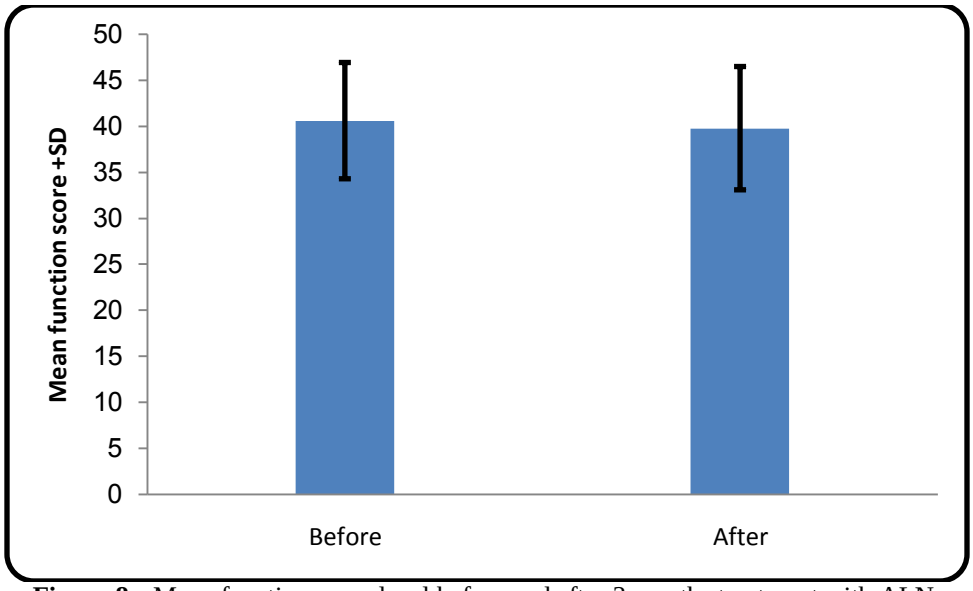

Figure 8:- Mean function score level before and after 3 months treatment with ALN.

Discussion:-

The mainstay of pharmacological therapy of OA is reducing pain and cartilage protection. Significant improvement in Western Ontario and McMaster Universities (WOMAC) scores together with no significant alteration in Kellgren-Lawrence grade (KL) joint space width (JSW) were stated by the current study. The present study showed that ALN use was associated with reduced knee pain severity as assessed by WOMAC scores. In agreement with our finding, Carbone and coworkers in 2004 reported significant reduction in pain according to WOMAC score in patients with OA after 3 years treatment with ALN. In agreement with current results, placebo control trail has found that there was a substantial improvement in total WOMAC score after ALN treatment and best improvement happened at week 4 (Jokar et al., 2010).Nishii and coworkers in 2013 suggested that 2 years ALN intake is effective in pain suppression associated with no significant alteration obtained in OA changes (radiological JSW)**.**Same advantages for bisphosphonate obtained in reducing pain symptoms were reported with clodronate (Rossini*et al.*, 2015) and risedronate (Spector *et al.*, 2005). Clifton and coworkers in 2006 demonstrated that there was no significant changes in WOMAC between the placebo and risedronate treated groups.

The current study demonstrated that changes in mean serum calcium, three months after treatment with ALN, were not significant despite minor reduction in calcium level. The early prevention of resorption of bone produces a decrease in serum calcium which resulted to raised parathyroid hormone (PTH), and eventually a rise in 1,25-dihydroxyvitamin D. The bisphosphonate induced secondary hyperparathyroidism also resulted in conservation of urinary calcium and phosphaturia, and a decrease in serum phosphate. The rise in the PTH after bisphosphonate treatment is a response to the variation in serum calcium and may happen even in the presence of hypercalcaemia, and this can lead to confusion in the explanation of PTH results (Vasikaran *et al*., 2001). This finding is go with the results were obtained by Heckbert and coworkers in 2008, Poole andcoworkers in 2007, Reid and coworkers in 2002 and Vasikaran and coworkers in 2001.

Regarding C-reactive protein (CRP), the current study showed an insignificant reduction in its level. It has been found that the two main acute-phase proteins reaction are C-reactive protein (CRP) and serum amyloid A, both of which can rise up to 1000 times over normal range following an acute phase reaction (APR) (Ceciliani *et al*., 2002). The pathogenesis of APR has been disclosed only recently. It has been hypothesized that intravenous bisphosphonate are taken up by endocytic cells, possibly monocytes or dendritic cells, resulting in the inhibition of farnesyl pyrophosphate (FPP) synthase. This inhibition results in a lack of geranylgeranylation and farnesylation of translated little guanosine triphosphate (GTP). GTPase participate with several essential cellular actions for survival. Moreover, the FPP synthase stoppage causes intracellular accumulation of isoprenyl pyrophosphate metabolites upstream of FPP synthase in the pathway of the mevalonate (Roelofs *et al*., 2006). Especially, this results in the aggregation of dimethylallyl pyrophosphate and isopentenyl pyrophosphate (IPP), that are powerful assists of the gama delta T-cell receptor (Galluzzo *et al*., 2007). They are naturally detected by gama delta T cells with eventual activation and liberation of TNF (tumor necroting factor)-beta, IFN (interferon) - gama and IL (interleukin) - 6, that are the proinflammatory cytokines enrolled in the initiation and maintenance of APR (Galluzzo *et al*., 2007).The current CRP result is in accordance with finding of Evio in 2006 who found no significant alteration in its CRP level in ALN treated osteoporotic patients and Bertoldo and coworkers in 2010 who stated that CRP shows significant rise within 2 days after intravenous infusion of bisphosphonate then it returns to normal levels.

No significant change in mean alkaline phosphatase (ALP) before and after treatment with ALN was reported in current study. Measurement of serum bone specific ALP may be used for monitoring bone anti-resorption treatment as the reduction in bone turnover is reflected in the form of reduced serum bone specific ALP (Kress, 1998); This may be explained by the fact that measuring serum total ALP may not reflect minor changes in bone specific ALP and also that patients in the current study had no significant osteoporosis. Failure of ALN in reducing bone resorption cannot be accepted as an explanation for the lack of the change of serum ALP because bone density has been shown to increase in studies carried out by Horikawa and coworkers in 2015 and Waikakul and coworkers in 2011. The current finding in agreement with Waikakul and coworkers in 2011, who studied the effect of ALN in patients with osteoporosis for 12 months; Horikawa and coworkers in 2015, who studied the effect of intravenous and oral treatment with ALN in patients with osteoporosis for one year. However, these results are in contradiction to the results obtained by Kress in 1998, who found that, in osteoporosis, there was highly significant reduction in bone specific alkaline phosphatase after three months ALN treatment. However serum total ALP did not show significant reduction neither in the present study nor in the studies carried out by Horikawa and coworkers in 2015 and Waikakul and coworkers in 2011.This is a good indicator that treatment with ALN did not affect liver metabolism.

Significant reduction in serum transforming growth factor beta 1(TGF-β1) levels following ALN treatment was made in the current study. It has been shown, in an experimental study, that TGF–β1 is activated in the subchondral bone as a response to chnaged mechanical force in an anterior cruciate ligament transection (ACLT) in mouse model with OA (Zhen et al., 2013). High levels of TGF–β1 caused the existence of nestin positive mesenchymal stem cell (MSC) clusters causing aberrant bone formation associated with increased neovascularization. Stoppage of TGF–β activity in subchondral bone reduced degeneration of OA joint cartilage (Zhen et al., 2013). It is worth to mention, deactivation of the TGF–β type II receptor (TβRII) in nestin-positive MSCs minimized process of OA in ACLT mice (Zhen et al., 2013). Thus, high levels of functioning TGF–β1 in the subchondral bone started the pathological events of OA, blockege of which may be a possible therapeutic approach (Zhen *et al*., 2013; Livshits *et al*., 2010).An experimental study has reported that induction of high level of TGF-β1 in bone morrow of mouse leading to abnormal bone remodeling, defects in the subchondral bone, including abnormality in bone mineral density and microstructure (Jiao *et al*., 2014). However, increase TGF-β1associated with abnormal reshaping of subchondral

bone which increase the possibility of progressive degradation of mandibular condylar cartilage and OA progression (Jiao *et al.*, 2014).Aberrant functioning of TGF β in the subchondral osseous tissue in response to an aberrant mechanical load induces development of osteroid islets at the starting of OA. Subsequently, modulation of subchondral bone structure varies the distribution of stress on the joint cartilage and causes its degeneration. Thus, stoppage of TGF-β activity in the subchondral osseous tissue may give a new approach for treatment of OA (Zhen*et al.*, 2014). Others, response to aberrant mechanical load, TGF-βs were liberated, stimulated and aggregated in subchondral bone to activate abnormal bone synthesis and neovascularization through recruitment of nestin-positive MSCs or osteoprogenitor cells throughout the pathological events of OA ; prevention of this process may be a possible therapeutic avenue to treat OA (Jie et al., 2014). In contrary to our results, it has been shown that ALN treatment increased serum TGF-β1 levels in experimental rat one year after treatment (Jia *et al*., 2013); however, other published articles showed that administration of ALN has markedly reduced TGF-β1 expression. So it is well obvious that reduction of TGF–β1 by ALN, as shown in the present study, is of great help in ameliorating OA.

The current study showed significant reduction in serum CTXII, a cartilage degredative marker. In agreement with Nishii and coworkers in 2013, the importance of reduction in CTX-II on suppression of cartilage degeneration confirmed by other studies (Willem*et al*., 2013 ; Dam*et al*., 2009) so the reduction in level of CTX-II in the present study may be considered as one of the mode of ALN action in OA.

Conclusions:-

The use of Alendronate in patients with osteoarthritis has clinical efficacy in reducing symptoms especially pain probably through inhibition of TGF beta 1with no structural improvement and may help delay and prevent further disease progression probably through inhibition of TGF β1 activity in the subchondral bone.The reduction in level of CTX-II in the present study may be considered as one of the mode of actions of alendronate in patients with OA.

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