IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 4 Ver. 17 (April. 2018), PP55-59
www.iosrjournals.org

Oxidative Stress in Osteoarthritis: Prolotherapy versus Traditional Management, a Comparative Study.

Kuladip Mukhopadhyay[1], Sagnik Mukherjee[2].
1. Associate Professor, Department of Orthopaedics, Midnapore Medical College.
2. Senior Resident , Department of Orthopaedics, Midnapore Medical College.
**Corresponding Author: Kuladip Mukhopadhyay*

***Abstract**: Introduction: Prolotherapy is an emerging option in the management of osteoarthritis though its mechanism of action is not well established. Oxidative stress plays a major role in the pathogenesis of osteoarthritis and we hypothesized that prolotherapy might mitigate oxidative stress in osteoarthritis.*
Aim: To study and compare the effect of prolotherapy and traditional management on oxidative stress and on follow up WOMAC score of the patients with osteoarthritis of knee.
***Materials And Methods:** Comparative study lasting 3 years on 37 known cases of primary and secondary osteoarthritis with 58 knees was performed. Patients with comorbidities or received any treatment previously were excluded. Patients were allocated to 2 groups using simple sequential randomization. First group (n=26) was injected with 3ml 25% dextrose intraarticularly and 3ml in the surrounding soft tissues on 0th, 4th and 8th weeks. Second group (n=32) underwent treatment with naproxen and glucosamine- diacerin combinations. WOMAC scores were calculated on 0th, 8th, 26th, 52nd and 104th week and synovial fluid was analyzed on 0th, 8th and 26th week for Total Antioxidant Status (TAS), Carbonyl compound status, Thiobarbituric acid reactive substances (TBARS) and total thiol compounds.*
***Results:** Complete follow up was possible for patients with 43 involved knees. Rise in oxidative stress on 8th week prolotherapy was statistically significant ($p<0.05$) compared to the baseline levels and 2nd group. At 26 weeks, oxidative stress parameters were comparable between the two groups ($p>0.05$) except total thiol status which was increased in the prolotherapy group ($p<0.05$). Statistically significant improvement in WOMAC scores were noted from baseline in both groups but not between themselves.*
Conclusion: Prolotherapy caused a significant increase in oxidative stress initially, by aseptic inflammation which seemed to trigger biological feedback pathway in vivo which caused intraarticular rise in total thiol (proxy marker for glutathione) and thus counteracted oxidative stress. Using antioxidants during prolotherapy might be counterproductive.

***Keywords:** Antioxidants, methyl glyoxal, Reduced glutathione, Thiobarbituric acid reactive substances*

Date of Submission: 16-04-2018 Date of acceptance: 02-05-2018

I Introduction

Osteoarthritis (OA) is the commonest joint disease affecting the human body and is an important cause of disability. It is characterized by focal cartilage loss with accompanying periarticular response in the form of subchondral bone sclerosis and attempted new bone formation in the form of osteophytes as a result of proteoglycan depletion[1]. Osteoarthritis has a prevalence of 330/10,000 in rural and 550/10,000 in urban India [2].

Prolotherapy, a term derived from proliferant or proliferation therapy, is an injection therapy for chronic musculoskeletal pathologies including knee osteoarthritis [3,4]. Studies by several authors including Rabago et al point to the significant improvement in patients in terms of pain control and also in imaging studies of the joint [3]. However, the mechanism of action of this modality is not well established.

Presence of elevated levels of oxidative stress parameters in osteoarthritis has been reported previously [5,6]. Reactive Oxygen Species (ROS) cause progression of osteoarthritis by cartilage ageing and chondrocyte senescence [7]. Study of Total Antioxidant status (TAS) quantifies body's defence against the oxidative damage in the synovial fluid [8,9].

The increased amounts of ROS attack the lipoproteins, deoxyribose moiety of DNA etc and produces Malone Di-Aldehyde(MDA) like compounds that is measured by estimating TBARS or thiobarbituric acid reactive substances [10].

Carbonyl stress affects osteoarthritis via OH. free radicals which are generated when Methyl Glyoxal binds with the amino groups of several amino acids [11,12] and its level provides key insights into the milieu of synovial fluid.

Glutathione, one of the thiol compounds is the substrate for the enzyme glutathione peroxidase, thereby regulating oxidative free radicals. Methyl glyoxal reacts spontaneously with glutathione and forms the hemimercaptal derivative, which is the substrate of the enzyme glyoxalase 1 [11]. So, GSH (Reduced glutathione) prevents methyl glyoxal accumulation in the body and reduce cellular damage.

Several studies have hinted at the possible beneficial role of prolotherapy in Osteoarthritis by initiating aseptic inflammation in the cartilages, which may be by modulating the oxidative stress pathways, but literature reviews fail to show any study trying to establish the relation between the two [3,4].

So the authors tried to study and compare the levels of oxidative stress parameters in osteoathritic knees treated by prolotherapy and those treated traaditionally with NSAIDS(Non Steroidal Anti Inflammatory Drugs)and conventional disease modifying Osteoarthritis drugs (DMOADS).

II Materials And Methods

A hospital based longitudinal comparative study was carried out in the Department of Orthopaedics, Midnapur Medical College, West Bengal from January, 2013 to December, 2015. After due approval of the Research Ethics Board of the institute, 37 newly diagnosed patients (46-85 years of age) with 58 knees (21 patients with bilateral affections and 16 with unilateral affection) affected by either primary or secondary osteoarthritis according to ACR criteria (Kellgren Lawrence grade 2 and 3) were included in the study [13]. The patients who had received any sort of treatment for their knees including intralesional steroids, viscosupplementation or Arthroscopic procedures were excluded from the study, as were the patients with comorbidities like diabetes mellitus or renal diseases or taking antioxidant medications which could influence the outcomes of the study.

These patients were then allocated to 2 groups by simple sequential randomization. Per protocol analysis was used. Initially, 26 knees (18 patients) were allotted to group A and 32 (19 patients) to group B. Group A patients were treated with 6ml intraarticular injection of 25% dextrose of which 3ml was injected intraarticularly in the knee joint by inferomedial or superolateral approach and remaining 3ml infiltrated around tendons and soft tissues. The injections were performed on 0th, 4th and 8th weeks. They were additionally given oral tramadol and paracetamol tablet for management of pain flare ups. The surrounding soft tissues injected included the patellar tendon, the medial and lateral collateral ligaments, the quadriceps tendon with 0.75ml injected in each of these structures. The aseptic inflammation is expected to cause influx of growth factors including platelet derived growth factor with resultant cellular hyperplasia which will reverse the degeneration of these tendons.

Group B patients were treated with oral NSAIDs (naproxen 500mg twice daily for 5 days and then on to control flare ups) followed by glucosamine and diacerein combinations (glucosamine 750 mg and diacerein 50 mg twice daily) for the rest of the period of study.

For both groups, lifestyle modification, exercises and bracing as and when required were allowed. Samples of 3ml synovial fluid were collected from the affected knee joints on 0th, 8th and 26th week for both Group A and Group B and analysed for oxidative stress markers as mentioned below. Initially the authors had proposed taping of synovial fluid on two more occasions (52^{nd} and 104^{th} week) but this was not approved by the Ethics Board. It was centrifuged at 5000 rpm and then stored at -20°C until final processing.

Study variables:-

(a) Total thiol status of the synovial fluid [14].

(b) Thiobarbituric Acid Reactive Substance (TBARS) of the synovial fluid [15].

(c) Carbonyl compound status of the synovial fluid[12].

(d) Total antioxidant status (TAS) of the synovial fluid [16].

(e)WOMAC score (Likert scale version) were calculated on 0th, 8th, 26th, 52nd and 104th Week [17]. WOMAC score is a validated questionnaire including weighted average of 3 subscale scores of knee pain, stiffness and function as assessed by the patient on a 0 to 100 scale.

Post injection protocol:- The patients were advised knee rest for 3 days following the injection with gradual return to normal activity over next 7 days. Static and isometric knee exercises were demonstrated, ice application was encouraged thrice daily with lifestyle modification like use of western toilets and compression bandages. The patients were followed up weekly for one month post injection and thereafter according to the protocol.

III Statistical Analysis: -

Statistical analysis was done with SPSS software (version 21.0). Mean and standard deviation of the samples were calculated and the level of significance calculation was done by paired t-test within the group and

independent t-test in between the groups. Results were considered significant if p values were less than 0.05 [95% confidence interval].

IV Results:

Of the 58 knees, 43 completed the follow up and their statistics were included in the statistical analysis according to the per protocol analysis methodology. The baseline characteristics of the two groups are described in table/ figure 1. In both the groups, most patients were aged more than sixty. Kolmogorov and Smirnov analysis showed comparability of the two groups.

Table/figure 1: Demographic constitution of the two groups.

Characteristic	Group A	Group B
No.of participants completing follow up [No. of patients (No. of knees)]	14(21)	13(22)
Age (Mean±SD) in years	68±7.8	65±8.6
No. of females	9	9
No. of years with symptoms (Mean±SD)	5.6±2.8	6.2±3.1
Kellgren-Lawrence score (No.of knees)	2(13) 3(8)	2(9) 3(13)
Baseline WOMAC score (mean± standard error)	66.64±1.07	65.29±1.16

The mean and standard error of the biochemical markers of oxidative stress in two groups is summarized in table/ figure number 2. There was a increase in antioxidants in group A which was not so well marked in group B. There was not much alteration in the carbonyl status in either of the groups.

Table/figure 2: Oxidative stress markers in the synovial fluid of group A and B patients expressed as mean± standard error of mean.

Marker	Week 0		Week 8		week 26	
Group	A	B	A	B	A	B
Total Thiols (µM)	439.86± 4.13	450.14± 5.62	339.76± 5.33	454.50± 4.67	566.14± 4.80	458.45± 4.49
TAS (mM)	2.146± 0.156	2.59± 0.113	1.317± 0.067	2.306± 0.121	2.345± 0.120	2.369± 0.086
Carbonyl compounds (nmol/ml)	75.762± 3.219	85.136± 2.582	88.428±3.287	82.500± 3.090	86.714± 2.208	83.454± 2.148
TBARS (nmol/ml)	1.582±0.022	1.597±0.026	1.894±0.023	1.543± 0.037	1.695± 0.047	1.543± 0.036

Table/ figure 3:Paired t-test significance values for Oxidative stress markers in the synovial fluid of group A patients at different weeks of therapy.

Marker	Week 0 vs week 8	Week 8 vs Week 26	Week 0 vs week 26
Total Thiols	p<0.05	p<0.05	p<0.05
TAS	p<0.05	p<0.05	p>0.05(p=.084)
Carbonyl compounds	p<0.05	p>0.05(p=0.668)	p<0.05
TBARS	p<0.05	p<0.05	p<0.05

Table/figure 4: Paired t-test significance values for Oxidative stress markers in the synovial fluid of group B patients at different weeks of therapy.

Marker	Week 0 vs week 8	Week 8 vs Week 26	Week 0 vs week 26
Total Thiols	p>0.05(p=0.476)	p>0.05(p=0.474)	p>0.05(p=0.162)
TAS	p>0.05(p=0.643)	p>0.05(p=0.511)	p>0.05(p=0.326)
Carbonyl compounds	p>0.05(p=0.344)	p>0.05(p=0.743)	p>0.05(p=0.571)
TBARS	p>0.05(p=0.220)	p>0.05(p=0.198)	p>0.05(p=0.998)

The results of paired sample t-Tests of the biochemical markers in Group A and group B are summarized in Table/figure 3 and Table/ figure 4 respectively. For group A, here is significant change in all the markers between the zeroeth(baseline) and eighth weeks, eighth and twentysixth week and zeroeth and twenty sixth week except for Total Antioxidant status between zeroeth and twenty sixth week and carbonyl status between eighth and twenty sixth weeks. No such significance is noted between the weeks in group B.

Table/ figure 5: The results of significance of the independent t-tests for the oxidative stress markers between the two groups: -

Marker	Week 0	Week 8	Week 26

Total Thiols	p>0.05 (p=0.15)	p<0.05	p<0.05
TAS	p>0.05(p=0.56)	p<0.05	p>0.05(p=0.882)
Carbonyl compounds	p<0.05	p>0.05(p=0.19)	p>0.05(p=0.296)
TBARS	p>0.05 (p=0.68)	p<0.05	p<0.05

Although all the markers showed significant difference between the two groups in eighth week, only total thiol levels were significantly different between the groups in 26th week.

Table/ figure 6:-The mean and standard error of the WOMAC scores for the two groups .

	Week 0	Week 8	Week 26	Week 52	Week104
Group A	66.64±1.07	69.73±1.12	66.53±0.82	60.44±1.12	60.06±1.01
Group B	65.29±1.16	64.33±1.32	62.33±0.95	62.32±0.92	62.56±1.32
Significance	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

Independent t test of significance has been used.
Table/ figure 6 summarizes the mean and standard error of the WOMAC scores for the two groups. There was statistically significant difference in the baseline WOMAC and WOMAC at 1 year and 2 year follow up in group A but independent sample t-tests between group A and group B failed to reveal any statistically significant difference.

V Discussion

The study of effect of prolotherapy on oxidative stress parameters was something novel and had not been described in literature so far. Our baseline level of TBARS in synovial fluid was comparable to that of 1.2±0.37nMol/ml found in a similar mongoloid population of South East Asia with knee osteoarthritis on antioxidant therapy by Angthong et al [18]. However, from our study, there appeared an initial flare up of oxidative stress after dextrose prolotherapy in the synovial joint. Jensen et al in their study had shown an initial rise of inflammatory markers in the knee ligaments in rat model after prolotherapy [19]. This was consistent with our findings since oxidative stress is an important part of the inflammatory responses via myeloperoxidase, fenton pathways and iNOS (inducible nitric oxide synthase) pathway [5]. However, at 26 week follow up, this oxidative stress flare seemed to resolve except for the carbonyl stress and accompanied by a concomitant increase in the total thiol status, which was a proxy marker for antioxidant potential of the body. There was significant increase in the total thiol status at the end of 26 week follow up in the Group A when compared to the baseline levels and also when compared to group B. So, the initial phase of oxidative flare seemed to activate the feedback system of the body by concentrating antioxidants in the joint most probably by overexpressing the gene for glutathione protein or glutathione peroxidise enzyme to counter the oxidative flare. This build-up of Total Thiols in general and glutathione in particular may help to mitigate the effects of the oxidative stress that might be the initiator of chondrocyte senescence by acting on the telomere of the DNA of chondrocytes, a chain of events that merges in the spectrum of osteoarthritis. So, from our study, dextrose prolotherapy seemed to improve the antioxidant potential of knee joints in osteoarthritis and this might be one of the mechanisms of its action. Also it seemed prolotherapy did not have a significant effect on the long run on carbonyl stress and hence on Advanced Glycation Endproducts. The effectiveness of prolotherapy in improving the composite WOMAC score in knee osteoarthritis has once again been affirmed. The result of our study was consistent with the results obtained by the Wisconsin study group of Rabago et al who had reported a progressive improvement over the 52 weeks of study [20]. The WOMAC score for group A had increased initially corresponding to the phase of initial aseptic inflammation followed by the improved phase corresponding to the build up of antioxidants. In group B (conventional treatment group), there was improvement of the WOMAC scores from the initial week but this became static after 24 weeks. The status of oxidative stress markers showed some improvement when analysed on the 8th and 16th week but there was no statistically significant improvement from the baseline. This improvement might be due to the antiinflammatory or the anti-chemotactic effect of the oral DMOADs. In between the two modalities, there was no statistically significant difference of the WOMAC scores as measured every week.

Limitations: - Our study relied heavily on patient response to quantify the improvement of the disease activity. No objective clinical or radiographic criteria were set to monitor disease activity. WOMAC score had taken into consideration the overall performance of bilateral knees rather than concentrating on the knee of interest. Also, it did not have the advantages of immunohistochemistry or molecular marker evidence to back up the findings. Due to the lack of appropriate modern kits which require financial assistance, older methods had to be used to quantify the markers. A gene marker or PCR evidence to identify the overexpression of gene for glutathione might have substantiated our study further.

Conclusions:- Our study reaffirmed that prolotherapy may be effectively used in the treatment of Kellgren Lawrence grade 2 and 3 osteoarthritis of the knee. Our study showed that prolotherapy seemingly acts

by increasing the levels of Total Thiols in the synovial fluid of the patients which modifies the oxidative stress component of osteoarthritis. As prolotherapy modifies the oxidative stress in the joints in a suitable way, antioxidants with prolotherapy is a promising option in the treatment of osteoarthritis of knee.

References:-

[1]. Peyron JG, Altman RD. The epidemiology of osteoarthritis.In: Moskowitz RW, Howell DS,Goldberg VM, et al, editors. Osteoarthritis: diagnosisand medical/surgical management. 2nd edition.Philadelphia: W.B. Saunders; 1992 :15–37.
[2]. Haq SA, Davatchi F. Osteoarthritis of the knees in the COPCORD world. Int J Rheum Dis.2011; 14(2):122-129.
[3]. Rabago D, Slattengren A, Zgierska A. Prolotherapy in the primary care practice. Prim Care.2010;37(1):65-80.
[4]. Reeves KD,Hassanein K. Randomized prospective double blind placebo-controlled study of dextrose prolotherapy for knee osteoarthritis with or without ACL laxity. AlternTher Health Me.2000;6(2):68-80.
[5]. Regan E, Bowler R, Carpo J. Joint fluid antioxidants are decreased in osteoarthritic joints compared to joints with macroscopically intact cartilage and subacute injury.Osteoarthritis and Cartilage.2008;16(4):515-521.
[6]. Maneesh M, Jayalekshmi H, SumaT,.Evidence for oxidative stress inosteoarthritis. Indian J Clin Biochem.2005.20(1):129-130.
[7]. Martin JA, Buckwalter JA. Roles of articular cartilage ageing and chondrocyte senescence in the pathogenesis of osteoarthritis. Iowa Orthop J.2001;21:1-7.
[8]. Merry P, WyniardP,Morris C, Grootveld M , BlakeD. Oxygen free radicals, inflammation, and synovitis : the current status. Ann. Rheum. Dis.1989;48(10): 864- 870.
[9]. Gutteridge J, Halliwell B. Antioxidants: Molecules,medicines, and myths. Biochemical and Biophysical Research Communications. 2010;393(4):561-564.
[10]. Afzal M, Armstrong D. Free Radicals in Biosystems. Molecular Biotechnology.2007;. 37(1):1-1..
[11]. Firuzzi O, Spadaro A, Spadaro C, RiccieriV, Petrucci R, Marrosu g et al. Protein oxidation markers in the serum and synovial fluid of psoriatic arthritis patients. J Clin Lab Anal. 2008;22(3):210-215.
[12]. Gilbert R, Brandt R. Spectrophotometric determination of methyl glyoxal with 2,4 dinitrophenylhydrazine. Analytical Chemistry. 1975;47(14):2418-2422.
[13]. Kellgren J, Lawrence J. Radiological assessment of osteoarthritis .Ann Rheum Dis.1957;16:494.
[14]. Hu ML, Measurement of Protein thiol groups and glutathione in plasma. Methods Enzymology 233: 1994: 382-385.
[15]. Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by Thiobarbituric Acid reaction. Annal Biochemical. 1979; 95.:351-358.
[16]. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C,Antioxidant activity applying an improved ABTS radical cation decolorization assy. Free Radical Biology and Medicine1999;26(9-10):1231-1237 .
[17]. Bellamy N, Buchanan W, Goldsmith C, Campbell J,Stitt L. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol. 1988;15(12):1833-1840.
[18]. Angthong C, Morales N, Sutipornpalangkul W, Khadsongkram A, Pinsornsak P, Pongcharoen B. Can levels of antioxidants in synovial fluid predict the severity of primary knee osteoarthritis: a preliminary study. SpringerPlus. 2013;2(1):652.
[19]. Jensen KT, Rabago D, Best TM, Patterson JJ, Vanderby R. Response of Knee Ligaments to prolotherapy in a rat injury model. Am J Sports Med.2008;36(7):1347-1357.
[20]. Rabago D, Patterson JJ, Mundt M, Kijowski R, Grettie J, Segal NA et al.Dextrose prolotherapy for knee osteoarthritis: A randomised controlled trial. Ann Fam Med. 2013;11(3):229-237.

Kuladip Mukhopadhyay." Oxidative Stress in Osteoarthritis: Prolotherapy versus Traditional Management, a Comparative Study.."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 4, 2018, pp 55-59