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CLINICAL ASSESSMENT OF EXPERIMENTALLY INDUCED OSTEOARTHRITIS RAT MODEL IN RELATION TO TIME

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ABSTRACT

Osteoarthritis (OA) is an age related joint disease manifested by progressive degeneration of articular cartilage and is associated with chronic pain. Dynamics of pain, lameness and swelling during developmental stages of experimentally induced OA in rat model. OA was induced in twenty five male Wistar rats by injecting papain (10mg/0.5cc buffer solution) intra-articularly and 0.9 per cent sterile saline solution in another group (n=5) of rats served as control. Clinical assessment based on pain, lameness and swelling was done on control group (day 0) and OA groups (n = 5) on 1st, 7th, 14th, 21st and 28th days post papain injection. Pain was recorded by hot water tail flick assay, degree of lameness by ordinal scoring system and swelling measured by calibrated digital caliper. Observations of OA groups were compared with of control group. Highest statistical mean retention time (3.43±.44) was observed on day 21st post papain injection, followed by day 14th (2.80±.59), 28th (2.46±.14), 7th (1.95±.47) and 1st (1.89±.47). Highest clinical score for swelling among osteoarthritis groups was determined on day one (6.31±.54), followed by day 07 (2.56±.28), 14 (2.30±.09), 21 (1.45±1.01) and 28 (1.14±.39), respectively. Highest lameness score was observed on 1st (2.40±0.55) and 14th day (2.40±0.55) followed by 21st day (2.20 ± 0.84), 7th day (1.60±.55). The least lameness score was observed on 28th day i.e. 1.40±.55. Highest cumulative mean clinical score observed was 10.38±1.10 (1st day) followed by 7.70±1.14 (14th day), 7.06±1.28 (21st day), 6.11±0.80 (7th day) and 5.00±0.34 (28th day) post papain injection. Clinical score was zero in control group. It was concluded that inflammatory signs of pain, lameness and swelling were reduced with passage of time in OA groups. This model may be used to study the efficacy of symptomatic slow acting/structure modifying drugs in pain management.

Key words: Osteoarthritis, papain, pain, lameness and swelling.

INTRODUCTION

Osteoarthritis (OA), a multi-factorial disease, is the consequence of both mechanical and biological events which in turn destabilize the normal process of degradation and synthesis of articular cartilage and subchondral bone. OA is the most frequent cause of physical disability among adults of age 60-65 years all over the world (Haq *et al.*, 2005). A combination of factors including overweight, aging, joint injury, stresses on joints in certain jobs and sport activities may lead to OA (Cooper and Coggon, 1999). Major signs and symptoms of OA include stiffness of affected joint and pain resulting in immobilization (Conaghan and Philip, 2008). Diameter of affected joints is more than normal. Affected joints are warm and there is loss of muscular bulk resulting in limited movement. Cartilage of the joints becomes tender and patient may feel crackling sounds on standing and walking. Commonly affected joints include knees, hips or hands (Brandt *et al.*, 2009). Characteristic OA features are seen in X-ray examination of joints (Lawrence *et al.*, 1966). However, pathophysiology of disease is complex and link to pain is poorly understood (Felson, 2005). Pain is the predominant clinical feature and treatment currently based on symptomatic relief of pain and swelling associated with OA to increase joint function. Non steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen are the most widely used drugs but cyclooxygenase-2 inhibitors, steroids and opiates may also be prescribed as well (Flood, 2010 Arroll *et al.*, 2004). Several OA models have been established to study pain related behavior (Bendele, 2001) and most commonly used model is mono-iodoacetate model (MIA) that is metabolic inhibitor of enzyme in chondrocytes resulting in disruption of glycolysis and eventually cell death (Guzman *et al.*, 2003). The disadvantage of this model is to produce robust morphological changes in articular cartilage coupled with severe pain and swelling that are not the characteristics of early phase of OA or initial degenerative phases of human cartilage. Etiological studies reveal that OA is age related problem that develops silently and progresses slowly. Patient feels pain and difficulty in movement on later stages of cartilage degeneration. So, there was a dire need of

developing model that may explain pain related behavior in early phase of OA resembling with spontaneous OA in human.

Despite the significant efforts has been consumed to develop models to understand pathogenesis of OA. Surprisingly little efforts were made to investigate pain related behavior associated with this disease. There is no study available where cumulative clinical scores were obtained from pain index, degree of lameness and extent of swelling in papain induced OA rodent model. This study narrates all above features recorded during developmental stages of OA in rodent model and its comparison with control group. It also gives information about time and concentration dependent use of papain model for study of anti-nociceptive effects of analgesics and anti-inflammatory agents.

MATERIALS AND METHODS

All experiments were carried out in accordance with the guidelines of the committee for Research and Ethical Issues of the International Association for the Study of Pain ® (IASP) and with institutional guidelines.

Twenty five male Wistar rats weighing 150-200g were selected from animal house of University of Veterinary and Animal Sciences, Lahore. The rats were housed (05 /cage) in a room with controlled temperature (21 to 22°C) and a reversed light/dark cycle (12/12 hours). Feed and water were provided *ad-libitum.*

Induction of osteoarthritis: OA was induced by injecting 10mg papain/0.5cc (Sigma, Cat # P 3125) in buffered solution of 0.05M sodium acetate, pH 4.5 with enzymatic activity of 31 I.U/mg intra-articulary in right knee joint of twenty five rats (pre-anesthetized with anesthetic ether) as described by the Murat *et al.* (2007). Five rats (n=5) were injected with 0.5cc of sterile saline solution (0.9%) in right knee joint that served as control group. Twenty five rats were divided into five groups (n=5) for development and assessment of OA. Clinical assessment based on pain, lameness and swelling was done on control group on day 0 and OA groups on 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days post papain injection (Janet *et al.*, 2004).

Pain assessment: Pain threshold was assessed in control and OA groups on days, 1^{st}, 7^{th}, 11^{th}, 14^{th}, 21^{st} and 28^{th} by the hot water tail flick assay as described by Hahn (1985). The dependent variable was the latency (in seconds) for the rats to flick its tail from the hot water bath. The water was maintained at 55^{o}C in a constant temperature water bath and monitored time to time by an electronic thermometer. Rats were wrapped in a breathable towel and gently held. The distal third of the rat's tail was dipped in water bath and time required for the rat to flick its tail from heat stimulus was counted with the help of stop watch. The tail flicking score was calculated as the mean of the last two of three readings separated by 30 seconds interval. Clinical score allocated for pain was one if tail retained in hot water for 0.1 to 1 seconds. Two clinical score for pain at retention time of 1.1 to 2 seconds, three for 2.1 to 3 seconds and four for retention time of 3.1 to 4 seconds respectively.

Degree of lameness: Degree of lameness in control and OA groups was recorded using ordinal scoring system. The scoring system was developed based on subjective scoring systems as reported in previous studies (Vasseur *et al.*, 1995; Holtsinger *et al.*, 1992). Degree of lameness was graded as mild, less moderate, moderate and severe with allocated scores of 01, 02, 03 and 04, respectively.

Measurement of swelling: Knee diameter was measured using calibrated digital caliper (world precisian Instruments, Stevenage, UK) in mm (millimeter) as described by Janet *et al.* (2004) to assess the developmental stages of OA with time interval in days.

Knee diameters were scored as 0.1-2mm as 01, 2.1-4.0 mm was allotted score 02, 4.1-6.0mm was allotted score 03 and 6.1-8.0mm was allotted score 04.

Cumulative clinical scores were also calculated by adding scores of pain, degree of lameness and swelling on each sampling day during development of osteoarthritis. Data was analyzed using SPSS version 13.0 by applying one way analysis of variance and compared by Duncan's Multiple Range (DMR) test at 95% probability.

RESULTS

Severity of pain was observed on the basis of retention time (in seconds) of tail in hot water for normal and OA induced rats. Statistical means were calculated for five replicates on day zero (pre papain injection) and days 1, 7, 14, 21 and 28 for groups which were injected with papain (OA groups). Highest statistical mean retention time (3.43±.44) was observed on day 21^{st} post papain injection, followed by day 14^{th} (2.80±.59), 28^{th} (2.46±.14), 7^{th} (1.95±.47) and 1^{st} (1.89±.47). Clinical score for pain in all OA induced rats differed significantly from normal group as analyzed by Duncan's multiple range (DMR) test. Rats observed on day 7 post papain injection were not significantly different from clinical scores observed at day 1 and 28. Osteoarthritis group on day 7 showed significant difference in clinical scores with other groups of other days. Observations of pain on days 14 and 21 were significantly different from each other and with clinical score of other groups (table 1).

Lameness was divided into four levels mild, less moderate, moderate and severe. Clinical scores for lameness were graded as 1, 2, 3 and 4 respectively. Statistical means of clinical scores for lameness observed

on days 1, 7, 14, 21 and 28 post papain injection differed significantly from that of normal group by DMR test. Highest clinical score for lameness was recorded in rats observed on days 1 and 14 post papain injection and were statistically non significant. Both observations differed significantly with findings of lameness on days 21 (2.20±.84), **7** (1.60±.55) and 28 (1.40±.55). Statistical mean values for lameness observed on day 7 were not significantly different from findings on 21 and 28 days as shown in table 1.

Swelling at the site of injection was measured in millimeters (mm) from day 0 (control) to 28th post papain injection (OA group). Swelling measured 0.1 to 2 mm was allocated clinical score of one (01) followed by 2.1 to 4.0 mm as two (02), 4.1 to 6.0 mm as three (03) and 6.1 to 8 mm as score (04). Clinical scores for swelling were calculated using five replicates on each day till 28th day. Highest clinical score among osteoarthritis groups

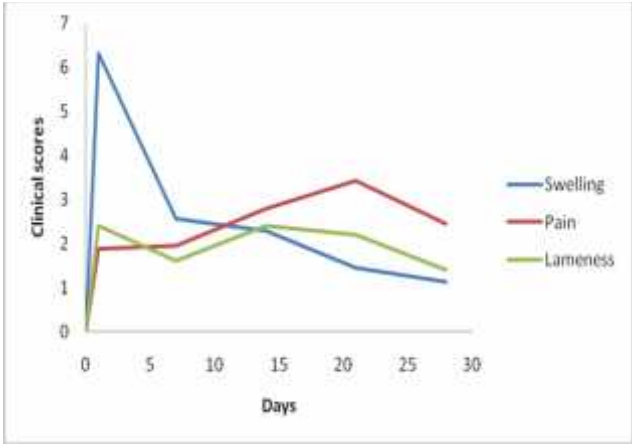

Figure 1: Curves for clinical scores including swelling, pain and lameness during development of OA on different days

Table 1. Clinical scoring for swelling, pain and lameness in experimentally induced OA rats on different days

Days	Replicates	Swelling mm	Mean ± S.D.	Pain (sec.)	Mean ± S.D.	Lameness	Mean ± S.D.	Total clinical score	Mean ± S.D.
Control	01	00		00		00		00	
Group	02	00		00		00		00	
(Day zero)	03	00	.00±.00^{a}	00	.00±.00^{a}	00	.00±.00^{a}	00	.00±.00^{a}
	04	00		00		00		00	
	05	00		00		00		00	
Osteoarthritis	01	6.23		2.61		2		10.84	
Groups	02	6.88		1.54		2		9.32	
Day 01	03	6.83	6.31±.54^{b}	2.12	1.89±.47$_{b}$	3	2.40±.55^{d}	11.95	10.38±1.1^{c}
	04	5.92		1.50		3		10.42	
	05	5.67		1.68		2		9.35	
Day 07	01	2.23		2.25		2		6.48	
	02	2.65		2.57		2		7.22	
	03	2.40	2.56±.28^{c}	1.78	1.95±.47$_{bc}$	2	1.60±.55$^{b}_{c}$	6.18	6.11±.80^{bc}
	04	2.97		1.31		1		5.28	
	05	2.55		1.86		1		5.41	
Day 14	01	2.32		2.05		2		6.37	
	02	2.28		2.94		2		7.22	
	03	2.44	2.30±.09^{c}	2.68	2.80±.59$_{d}$	2	2.40±.55^{d}	7.12	7.70±.1.14^{d}
	04	2.21		3.67		3		8.88	
	05	2.26		2.66		3		8.92	
Day 21	01	2.04		2.70		3		7.74	
	02	2.46		3.51		3		8.97	
	03	0.17	1.45±1.01^{d}	3.64	3.43±.44$_{e}$	2	2.20±.84$^{c}_{d}$	5.81	7.06±41.28$^{c}_{d}$
	04	0.56		3.86		2		6.32	
	05	2.00		3.46		1		6.46	
Day 28	01	1.28		2.29		1		4.57	
	02	1.69		2.47		1		5.16	
	03	0.84	1.14±.39^{d}	2.61	2.46±.14$_{cd}$	2	1.40±.55^{b}	5.45	5.00±.34^{b}
	04	0.69		2.35		2		5.04	
	05	1.20		2.58		1		4.78	

Mean±S.D with same superscripts differ non-significantly where as numeric values with different superscripts vary significantly.

was determined on day one (6.31±.54), followed by day 07 (2.56±.28), 14 (2.30±.09), 21 (1.45±1.01) and 28 (1.14±.39), respectively. All of the clinical scores for swelling calculated on different days differed significantly from normal group as compared by DMR using SPSS version 10.0. However, difference in clinical scores of swelling on days 7 and 14 was not statistically significant but significant with scores of days 1st, 21st and 28th ($P \leq 0.05$). Similarly, the difference in clinical score of swelling was not significant between days 21st and 28th but significant with score of other days (table 1).

Sum of swelling, pain and lameness scores was calculated to be used as total clinical score of OA groups on different days and analyzed statistically by DMR test. Highest mean clinical score observed was 10.38±1.10 (1st day) followed by 7.70±1.14 (14th day), 7.06±1.28 (21st day), 6.11±0.80 (7th day) and 5.00±0.34 (28th day) post papain injection. Clinical score was zero in normal rats. Results are presented at table 1. Results of pain, lameness and swelling in relation to time in the form of curve are attached as figure 1.

Clinical scores on days 7 and 28 did not differ statistically but differ significantly with clinical scores of all other days. Clinical score of day 7 was not statistically different from scores on day 21. Significant difference was observed on days 0 (normal), 1st and 14th. There was no significant difference statistically in clinical scores of days 14th and 21st but significant with other days. Clinical score on day one differed significantly with scores on all other days. It is concluded that inflammatory signs like redness, swelling, pain and loss of function can be related with the experimental OA. By calculating scores depending on the severity of clinical signs progression of the diseases can be assessed.

DISCUSSION

Different parameters were used to evaluate successful induction of OA in experimental models. Clinical signs, biochemical profiles, biomarker concentration in serum/urine/synovial fluid, histological findings, radiography and molecular techniques had been carried out in number of experiments for the evaluation of developed OA. In present study evaluation was based on clinical signs including pain, lameness and swelling in relation to time after papain injections in knee joint of rats under experimental conditions.

Clinical signs included were extent of swelling, pain and lameness on day 0 (control) and 01, 07, 14, 21 and 28th days post papain injection. Depending on the severity of clinical signs, scores were allocated which were obtained on different observational days. Highest mean clinical score for swelling on injected knee was observed on day one (6.31), followed by day 07 (2.56), 14 (2.30), 21 (1.45) and 28 (1.14), respectively. Swelling at the site of papain injection decreased with the passage of time and minimum on 28th day. Hot water tail flick assay was used for pain assessment on different days. Maximum pain was observed on 21st day (3.43), followed by 14 (2.80), 28 (2.46), 7 (1.95) and 1 (1.89) days post papain injection. There was gradual rise in pain till day 21st and then reduction in pain started. Third sign observed was lameness which was based on position of posture and utility of the joint during walk. Again converted to scores for quantification. Highest mean clinical score for lameness was recorded on days 1st, 14th and lowest on 28th (1.40) post papain injection. Clinical signs were countered by the natural immune system of rats but severity was observed around days 21st and 28th.

Highest mean clinical score (sum of swelling, pain and lameness scores) observed was on 1st day (10.38) followed by 14th (7.70), 21st (7.06), 7th (6.11) and 28th (5.00) days post papain injection in Wistar rats. Clinical scores on days 7 and 28 were not different statistically to each other but differed significantly with clinical scores on all other days. Clinical score of day 7 was not statistically different from scores on day 21st. There was no significant difference statistically in clinical scores of days 14 and 21 but significant with others. Clinical score on day one differed significantly with scores on all of the other days. Mean clinical score for control group was taken as zero that was significantly different from all other observations recorded on different days of post papain injection in rats.

Malfunctioning of knee joint is directly linked to the mild to severe damage to the anatomical structure. Responsible lesions could be aggregation of osteophytes (Creamer *et al*. 1999), Oedema around bone and inflammation of synovial membrane (Felson *et al*. 2001; Hill *et al*. 2001). Sowers *et al*. (2003) and Link *et al*. (2003) did not confirm the findings. Saxne *et al*. (2003) correlated swelling of knee post injection of chemical with initiation of synovial inflammation. Pressure of swelling on the free nerve endings (Buma *et al*. 2000) play key role for sensation of pain (Saito and Koshino, 2000). According to one concept, sensation of pain is related to increased afferent activity and lowered level of threshold for spinal nerves. Fernihough *et al*. (2004) included extent of swelling around knee as an index of inflammation. OA patents showed hyper analgesic responses to mechanical stimuli applied in the vicinity of knee joint (Bajaj *et al*. 2001). Allodynia in areas adjacent to the OA had been reported by Kosek and Ordeberg (2000). Creamer *et al*. (1999) studied pain in rats not only during motion but 43 percent had pain at rest as well. Relation between OA and presence of knee pain was established by Hannan *et al*. (2000). Association of pain with knee stiffness, thickening of synovial membrane and cartilage was reported by Wluka *et al*. (2004).

Findings of the present study in accord with others suggested that relationship of OA existed with swelling of knee joint, lameness and pain along with

other mechanical stimuli. Clinical signs can be used as an indicator of OA in various experimental models having OA (Van der Kraan *et al*. 1989; Guzman *et al*. 2003). Behavior changes due to pain in experimentally induced OA had not been explored at molecular level and the mechanism involved is still under discussion (Bove *et al*. 2003; Combe *et al* 2004). Limitations in the assessment of pain in OA are there but advantages to relate this with the degree of OA can be developed. Oestergaard *et al*. (2006) calculated macroscopic severity scores 15th day post induction of OA and declared disease onset on the basis of inflammation on investigated joints. Initial inflammatory response was observed by Bove *et al*. (2003) following the injection of monosodium iodoactate in knee joint is an effort to induce OA. Hendiani *et al*. (2003) used von Frey filaments as a tool to assess pain and in this sensational threshold was produced by mechanical way in chronic arthritis. Kean *et al*. (2004) had also reported relation of pain with OA. McCarthy *et al*. (2007) developed subjective scoring system based on different grades of lameness, joint mobility, pain on palpation, weight bearing and overall score of clinical conditions to assess the effectiveness of GS and CS in dogs with OA and then compared with carprofen (NSAID).

Fernihough *et al*. 2004 developed OA in two groups of Wistar rats by chemical and surgical means and extent of swelling of infected knee joints were measured by using calibrated digital caliper that is in accordance with the present study. Mechanical hyperalgesia was measured using electronic analgesy meter.

On the basis of findings of the present work and reported literature, it is concluded that inflammatory signs like redness, swelling, pain and loss of function can be related with the experimental OA. By calculating scores depending on the severity of clinical signs progression of the diseases can be assessed. Modes of measuring the disease severity vary and are the choice of the researcher. Both mechanical and physiological parameters can be selected to determine the extent of OA.

This model may be used to study the role of slow acting/structure modifying drugs in pain management. It may also be effective for pathophysiological studies of diseased bone, synovium and cartilage and their link with pain, lameness and swelling. Moreover it also explains that collagen degradation is not only the cause of pain and swelling in OA, there are many other factors that contribute towards severe pain during mobility of joints as in this model pain did not persist with same threshold. It may be due to active immune system of rodent to resist the inflammatory changes.

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