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Research Article

Piriformis Stretch and Muscle Activation for Sacoiliac Joint Pain



# The Combined Effectiveness of Piriformis Stretch and Muscle Activation Exercises in Patients with Sacroiliac Joint Pain.



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Abstract: Sacroiliac joint pain is one of the common misdiagnosed orthopedic causes of low back pain, which affects between 15 to 30 percent of individuals with mechanical low back pain below L5. The sacroiliac joint is found out to be a source of dysfunction and pain in 10% to 27% of suspected cases of patients with chronic low back pain. Clinicians use different electrotherapy modalities, biomechanical correction techniques, and exercises for addressing SI joint pain. Many studies have been undertaken to find out the efficacy of different treatment tools in combination or isolation. Likewise, there are studies done to understand the effect of piriformis stretch and muscles activation exercises on sacroiliac joint pain but, there exists very little evidence studying the combined effect of both the treatment tools. So our study aims to determine the combined effect of piriformis stretch and muscles activation exercises in patients with sacroiliac joint pain. About 30 subjects, both male and female with a primary diagnosis of sacroiliac joint pain by the physician were recruited into two groups. Group A having 15 patients received piriformis stretch and muscles activation exercises, and Group B having 15 patients received only muscles activation exercises. All the subjects received therapy sessions thrice weekly for four weeks. Outcome measures used were the visual analog scale (VAS) and oswestry disability index(ODI). Preintervention assessment and post-intervention assessment was carried out for both groups and the received data was analyzed using paired and independent t-test. According to the results of the analysis, the average improvement of VAS for Group A and Group B were 2.33 and 3.53 respectively. The paired t-test was 23.129 and 14.270, which is statistically highly significant (p-value = 0.000). The average improvement in oswestry disability index for group A and group B were 16.13 and 26.13 respectively using mean and standard deviation. The paired t-test was 11.014 and 6.934 respectively, which is statistically highly significant (p-value = 0.000). There is a significant improvement in both Group A and Group B. However, Group A (piriformis stretch and muscle activation exercises) showed significant improvement in the treatment of sacroiliac joint pain. The results indicated that both Group A and Group B had significant improvement in the scores of VAS and ODI scores at the 4th week when compared to baseline values, but when comparing the end results of group A and group B, it has been found out that group A intervention is more effective than Group B in treating patients with sacroiliac joint pain. From the data analysis reports, it can be concluded that when these treatment tools i.e piriformis stretch and muscles activation exercises are applied in combination, it gives better results in the management of sacroiliac joint pain

Keywords: sacroiliac joint pain, piriformis stretch, muscles activation exercises, oswestry disability index, visual analog scale, low back pain

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#### I. INTRODUCTION

The sacroiliac joint (SII), the largest axial joint in the body, connects the spine to the pelvis, which allows load transfer between the lumbar spine and the lower extremities. The SII lies between the sacrum and the ilium, spanning about 1 to 2 mm in width and the joints on either side of the sacrum is held together by a fibrous capsule. The sacroiliac joint being the largest joint in the body, with a surface area of approximately 17.5 cms is relatively immobile, and its primary function is to transfer weight to and from the lower limbs to the axial skeleton. There are three large lever arms, the trunk, and the lower limbs whose movements transmit significant force through the sacroiliac joint. The bony contours and the strong interconnecting ligaments allow only minimal motion at the joint surfaces<sup>2</sup>. Low Back pain due to Sacroiliac joint (SIJ) involvement, which is a frequently misdiagnosed cause, affects about 15% to 30 % of the common population. Pain below L5 where the Sacroiliac joint is present happens to be the source of pain very often in the suspected cases with chronic low back pain<sup>3</sup>. Usually, sacroiliac joint pain follows a three stage pattern of onset4, stage one is the new-onset/acute phase - (dysfunction) having a duration of I to 4 weeks, characterized by minor pathology causing abnormal motion and resultant pain. Stage 2 is the subacute phase- (instability) having a duration of I to 3 months. In this stage, there is a further disruption in SI joint stability with potential gait dysfunction and persistent pain. Stage 3 is the chronic/stable phase (stabilization) having a duration of more than 3 months. Here degenerative changes can occur and may manifest as sclerosis, joint erosion, and eventually ankylosis. The potential causes of sacroiliac joint pain can be either traumatic or atraumatic.<sup>5</sup> Radiographs are still used as the first-line imaging of the sacroiliac joints. The irregular outline and obliquity make the sacroiliac joints difficult to fully assess radiographically. This limitation has been overcome by more sensitive imaging techniques such as computed tomography (CT) or MRI. Ultrasound is not as useful in assessing the sacroiliac joints as only the anterior and posterior margins are seen. CT is excellent for detecting erosions, bone sclerosis, ankylosis and for guiding interventional procedures although MRI is superior in detecting bone marrow edema as a measure of sacroiliac joint inflammation. 7,8 NSAIDs (non-steroidal antiinflammatory drugs) are usually considered as the first-line medication for SIJ pain. A Cochrane systematic review of 51 RCT showed that patients with acute low back pain had symptomatic pain relief with NSAID treatment9 A variety of treatments have been recommended, physiotherapy demonstrating successful results like treatments for SIJ dysfunction usually emphasize the abdomino-lumbo-sacro-pelvic-hip complex. The treatments include: stretching, strengthening, stabilizing pelvic floor muscles, correcting gait abnormalities, and addressing postural and dynamic muscle imbalance. An exercise program includes core strengthening and for SII dysfunction correction of muscle imbalances that may increase the shearing forces in one SIJ. 10 Studies done on both sacroiliac joint syndrome (SIJS) and piriformis syndrome (PS) have highlighted that there is a relationship between SIJS and PS. Besides the referral patterns of the pain, they concluded that the symptom complex of PS is similar to that of the sacroiliac syndrome. 11 In a case study, a female patient with left low back pain and sacroiliac joint pain was managed with therapeutic exercise. The patient was seen six times to correct pelvic position/posture and left hip posterior capsule

restriction via (1) muscle activation (left hamstrings, adductor magnus, and anterior gluteus medius) and (2) left hip adduction to lengthen the left posterior capsule/ischiofemoral ligament. Stabilization exercises were included for bilateral hamstrings, gluteus maximus, adductors, and abdominals to maintain pelvic position/posture. The patient's pain was eliminated 13 days after she first performed three exercises to reposition the pelvis and restore left posterior hip capsule extensibility and internal rotation. The author concluded that the interventions to restore and maintain the optimal position of the pelvis and hip (femoral head in the acetabulum) may be beneficial for treating patients with chronic LBP/SIJP. 12 Likewise, the present literature shows here are various studies done to understand the effect of Piriformis stretch<sup>32</sup> and muscles activation exercises<sup>12</sup> on SI Joint pain but, there exists very little evidence studying the combined effect of both the treatment tools. So the study aimed to determine the effect of both piriformis stretch and muscles activation exercises in combination for the management of patients with sacroiliac joint pain.

# 2. MATERIALS AND METHODS

An experimental (comparative) study was conducted for 12 months. In this study, 30 patients pre-diagnosed by physicians fulfilling the inclusion criteria were recruited and randomly divided into two groups namely group A (experimental) and group B (control). Inclusion criteria included both male and female patients diagnosed with SI joint pathology within the age group of 45 to 55 years, pain over the sacroiliac joint with no surgical history or any history of trauma on the SIJ complex. Exclusion criteria included fracture in and around the SIJ Complex, tumor around the joint, arthritis of joint, patient not willing to participate in the study, and any history of surgery. The study proposal has been accepted by the Ethics Committee, Assam down town University (Memo No: adtu/Ethics/stdntlett/2021/08). Written consent was taken from the patients for participating in the study. The samples were collected from Assam Down Town University OPD and Physiotherapy Department, Down Town Hospital.

# 2.1 Outcome Measures

VAS<sup>19</sup> and ODI<sup>20</sup> were used for assessing pain and functional ability.

### 2.2 Procedure

In this study 30 subjects fulfilling the inclusion criteria were randomly divided into two groups, Group A (experimental group) and group B (control group), each group contained 15 subjects. A convenient sampling method was used to avoid the consequences of drop out of subjects and any further difficulty in carrying out the research. Group A subjects received piriformis stretch and muscles activation exercises 3 times a week for 4 weeks. Group B subjects did the muscles activation exercises 3 times a week for 4 weeks. For each subject, demographic data were collected and a Pre-intervention and Post-intervention assessment were carried out for both Group A and Group B by VAS for assessing pain and ODI for assessing disability, and data thus recorded were analyzed statistically using paired-t-test and t-test.

# 2.3 Piriformis Stretch

The subjects were made to bent two legs in a supine position and kept the leg of one side on the opposite side knee that would be measured (Fig 4.1.A). The subjects bent their knee

over 90° until they felt tension in the direction toward the shoulder on the same side as the leg that was being stretched and then maintained the position for 30 seconds (Fig 4.1.B).

This was repeated twice with a 30-second resting time in between. 13



Fig 4.1.A (Piriformis Stretch)



Fig:4.1.B (Piriformis Stretch)

# 2.4 Muscles Activation Exercises:

For activation of the adductor magnus, patient need to perform "sit to stand" position on a chair. For activation of gluteus medius and hamstrings muscle, the patient was asked to lie on the couch and was asked to perform certain standard exercises which are taught by the therapist with the frequency of 10 repetitions 3 sets of every exercise.

# 2.5 Muscles Activativation\_ 14

(left hamstrings, adductor magnus, and gluteus medius)

# 2.5.1 Adductor Magnus Activation,

Patient was asked to perform the close chain, weight-bearing task- sit to stand, to activate the Adductor Magnus (Fig 4.2.1C,4.2.1D)<sup>15,18</sup>







FIG4.2.1 C, 4.2.1 D (sit to stand Adductor Magnus Activation )

# 2.5.2 Gluteus Medius Activation

Patient was asked to perform bridging on a stable surface, to activate the gluteus medius. (FIG 4.2.2 E)<sup>16</sup>



FIG4.2.2 E (Bridging, Gluteus Medius Activation)

# 2.5.3 Hamstring Activation

The patient was asked to do a glute-hamstrings raise, to activate the Hamstrings. (FIG 4.2.3F)<sup>17</sup>



Fig 4.2.3 F (Glute-hamstrings raise, Hamstring Activation)

#### 5 Statistical Measures

Descriptive statistical analysis was carried out in the present study. Outcome measurements analyzed are presented as mean( $\pm$ )SD. Significance is assessed at a 5% probability level of significance with a p-value was set at 0.05 less than this is considered as a statistically significant difference. Using

purposive sampling; the t-test was used to analyze the variables pre-intervention to post-intervention with calculations of the percentage of change. The Statistical software namely SPSS16.0 was used for the analysis of the data and Microsoft Word 2007 and Excel 2007 have been used to generate the graph, tables, eDistribution of demographic variables

#### **5.1.a** Age

Table 5.1.a: Distribution of demographic variables(Age)									
N Minimum Maximum Mean									
Piriformis Stretch and Muscle Activation Exercises	15	38.00	61.00	48.93	3.61				
Muscle Activation Exercises	15	37.00	62.00	49.26	3.62				

The table 5.1.a shows that the average age of the patients under Piriformis Stretch and Muscle Activation Exercises treatment was 48.93 with a standard deviation of 3.61. The average age of the patients under Muscle Activation Exercises treatment was 49.26 with a standard deviation of 3.62.

# **5.1.b** Sex

Table 5.1.b: Distribution of demographic variables (Sex)									
	Piriformis Stretch and Muscle Activation Exercises Muscle Activation Exercises								
	Frequency	Percent	Frequency	Percent					
Female	8	53.3	8	53.3					
Male	7	46.7	7	46.7					
Total	15	100.0	15	100.0					

The table 5.1.b gives the frequency distribution of the patients. There were 53.3% female and 46.7% male patients in each group.

# 5.1 Joint Pain and disability improvement by Piriformis Stretch and Muscle Activation Exercises.

# 5.2.a. Improvement on VAS

Table 5.2.a: Improvement on VAS when received Piriformis Stretch and Muscle Activation Exercises for SI joint pain.									
	Mean	N	Std. Dev	t	df	р			
<b>Before Treatment</b>	6.73	15	1.38						
After Treatment	2.33	15	1.11	23.129	14	0.000			

Table 5.2.a is constructed to see whether piriform stretch and muscle activation exercises can improve joint pain concerning VAS. Paired t-test was performed to see the significant difference in VAS scores before and after treatment. It was found that t = 23.129 which

is highly statistically significant at a 1% probability level (p=0.000). We can say that there has been a remarkable decrease in VAS scores after treating the patients with piriformis stretch and muscle activation exercises.

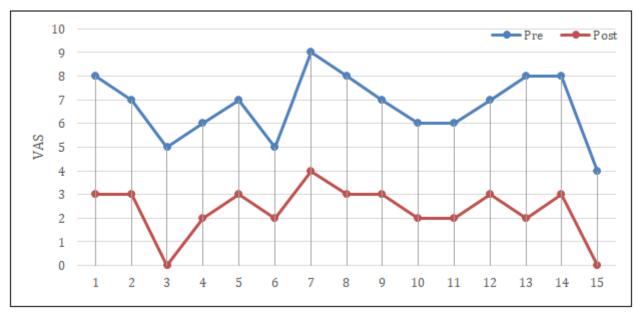


Fig 5.2.a: VAS scores of patients before and after treatment of piriformis stretch and muscle activation exercises

# 5.2.b. Improvement on ODI

Table 5.2.b: Improvement on ODI VAS when received										
Piriformis Stretch and Muscle Activation Exercises for SI joint pain.										
	Mean	N	Std. Dev	t	df	р				
<b>Before Treatment</b>	40.80	15	9.12	_						
After Treatment	16.13	15	2.06	11.014	14	0.000				

Table 5.2.b is constructed to see whether piriformis stretch and muscle activation exercises can improve joint pain with respect to ODI. Paired t-test was performed to see the significant difference in ODI scores before and after treatment. It was found that t=11.014

which is highly statistically significant at a 1% probability level(p=0.000). We can say that there has been a remarkable decrease in ODI after treating the patients with piriformis stretch and muscle activation exercises.

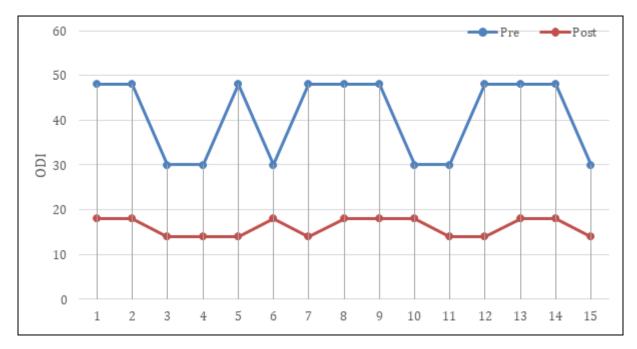


Fig 5.2.b:ODI scores of patients before and after treatment of piriformis stretch and muscle activation exercises

# 5.2 Joint Pain and disability Improvement by Muscle Activation Exercises

# 5.3.a. Improvement on VAS

Table 5.3.a: Improvement on VAS when received Muscle Activation Exercises for SI joint pain.										
	Mean	N	Std. Dev	t	df	р				
<b>Before Treatment</b>	6.20	15	1.32							
After Treatment	3.53	15	1.30	14.270	14	0.000				

Table 5.3.a is constructed to see whether muscle activation exercises can improve joint pain with respect to VAS. Paired t-test was performed to see the significant difference in VAS scores before and after treatment. It was found that t = 14.270

which is highly statistically significant at a 1% probability level (p=0.000). We can say that there has been a remarkable decrease in VAS scores after treating the patients with muscle activation exercises.

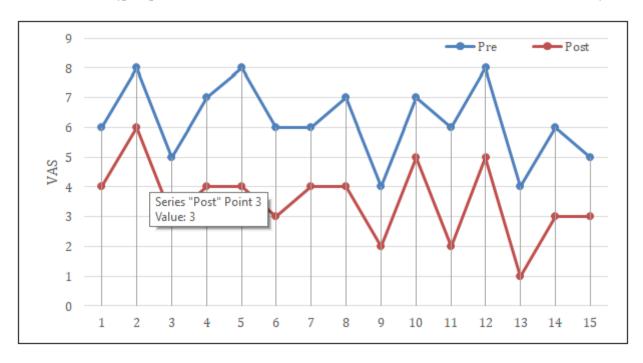


Fig 5.3.a: VAS scores of patients before and after treatment of muscle activation exercises

# 5.3.b. Improvement on ODI

Table 5.3.b : Improvement on ODI when received  Muscle Activation Exercises for SI joint pain									
	Mean N Std. Dev t					р			
<b>Before Treatment</b>	40.13	15	10.37						
After Treatment	26.13	15	3.15	6.934	14	0.000			

Table 5.3.b is constructed to see whether muscle activation exercises can improve joint pain with respect to ODI. Paired t-test was performed to see the significant difference in ODI scores before and after treatment. It was found that t = 6.934

which is highly statistically significant at a 1% probability level (p=0.000). We can say that there has been a remarkable decrease in ODI after treating the patients with muscle activation exercises.

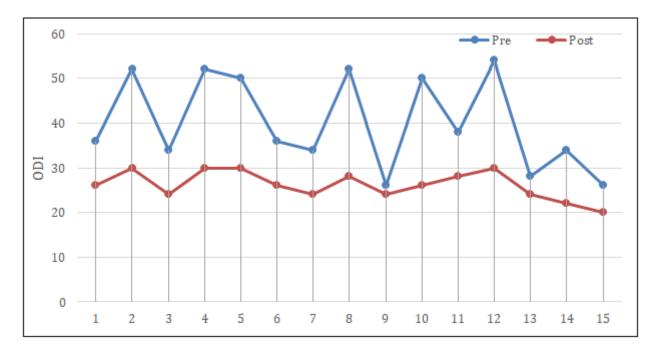


Fig 5.3.b: ODI scores of patients before and after treatment of muscle activation exercises

5.4.a. Comparison of VAS scores between the patients in the two groups PRE-TREATMENT.

Table 5.4.a: Comparision of VAS scores betweenthe patients in the two groups PRE- TREATMENT.									
	N	Mean	Std.Dev. t	df	р				
Piriformis Stretch and Muscle Activation Exercises	15	6.73	1.38	1.079	28	.290			
Muscle Activation Exercises	15	6.20	1.32						

Table 5.4.a gives the result of the t-test performed on VAS between the two groups of patients i.e. patients with joint pain treated by piriformis stretch and muscle activation exercises and patients with joint pain treated by muscle activation

exercises before treatment. t = 1.079 which is statistically not significant (p=.290). It has been inferred that there was no significant difference in VAS in the two groups before treatment.

# 5.4.b. Comparison of the effectiveness of piriformis stretch and muscle activation exercises over muscle activation exercises to decrease VAS scores POST TREATMENT.

Table 5.4.b: Comparison of the effectiveness of piriformis stretch and muscle activation exercises over muscle activation exercises to decrease VAS scores POST TREATMENT									
	N	Mean	Std. Dev.	T	df	р			
Piriformis Stretch and Muscle Activation Exercises	15	2.33	1.11	-2.714	28				
Muscle Activation Exercises	15	3.53	1.30	_		.011			

Table 5.4.b gives the result of the Independent t-test which was performed to compare the VAS scores of the patients with joint pain after treating with piriformis stretch along with muscle activation exercises and patients with joint pain treated with muscle activation exercises. t = -2.714 which is statistically significant at 5% probability level (p=0.011). It

has been inferred that VAS was seen to have decreased significantly among patients treated with piriformis stretches along with muscle activation exercises as compared to patients treated with only muscle activation exercise implying that piriformis stretches along with muscle activation exercises is more effective to reduce joint pain.

# 5.5.a. To compare ODI scores between the patients in the two groups PRE-TREATMENT

Table 5.5.a: Comparision of ODI scores between the patients in the two groups PRE-TREATMENT									
	N	Mean	Std. Dev.	t	df	p			
Piriformis Stretch and Muscle Activation Exercises	15	40.80	9.12	<del>-</del> 0.187	28				
Muscle Activation Exercises	15	40.13	10.37	- 0.187		.853			

Table 5.5.a gives the result of the t-test performed on ODI between the two groups of patients i.e. patients with joint pain treated by piriformis stretch and muscle activation exercises and patients with joint pain treated by muscle activation

exercises before treatment. t = 0.189 which is statistically not significant (p=.853). It has been inferred that there was no significant difference in ODI in the two groups before treatment.

# 5.5.b. Comparison between the effectiveness of piriformis stretch and muscle activation exercises over muscle activation exercises to decrease ODI scores POST TREATMENT

Table 5.5.b: comparison of the effectiveness of piriformis stretch and muscle activation exercises over muscle activation exercises to decrease ODI scores POST TREATMENT								
	N	Mean	Std. Dev.	t	df	р		
Piriformis stretch and muscle activation exercises	15	16.13	2.06	<del>-</del> -10.261	20			
Muscle activation exercises	15	26.13	3.15	-10.201	28	.000		

Table 5.5.b gives the result of the Independent t-test which was performed to compare the ODI scores of the patients with joint pain after treating with piriformis stretch along with muscle activation exercises and patients with joint pain treated with muscle activation exercises. t = -10.261 which is statistically significant at a 1% probability level (p=0.000). It has been inferred that ODI was seen to have decreased significantly among patients treated with piriformis stretches along with muscle activation exercises as compared to patients treated with only muscle activation exercises implying that piriformis stretches along with muscle activation exercises if more effective to reduce joint pain.

#### 6 DISCUSSION

Sacroiliac joint dysfunction (SIJD) is one of the major contributing factors to low back pain accounting for approximately 16%–30% of cases<sup>25-27</sup>. The SIJD generally

refers to the aberrant position or movement of sacroiliac joint (SII) structures that may or may not result in pain. Pain arising from the sacroiliac joint is one of the potential causes of axial low back pain. A quarter of low back pain could be originating from the sacroiliac joint<sup>24</sup>. Once the SII pathology is suspected, customized treatment plan should be framed which will best suit each individual patient. Apart from NSAIDs and muscles relaxants for concomitant muscle spasm, noninvasive treatments such as exercise, manual medicine, and orthotics like SIJ belts must be considered as options for controlling a patient's pain. If these management protocols fails, then procedures such as fluoroscopic, ultrasound, or computed tomography-guided SIJ injections and radiofrequency denervation should be considered with SIJ fusion being a last resort option.31Although there were episodes of failure of conservative managements of SIJP, there are quite a good number of literature that portrays a good response. Many physiotherapy interventions also have

been proved to be effective. This statement is backed by several studies done by different clinicians in which they studied the effectiveness of physical therapy interventions in the treatment of sacroiliac joint dysfunction (SIID) and concluded that physiotherapy interventions are effective in reducing pain and disability associated with SIJD.21 In a comparative study done between exercise therapy (ET), manipulation therapy (MT) in terms of their effectiveness in treating Sacroiliac joint dysfunction, it was concluded that Exercise and manipulation therapy appear to be effective in reducing pain and disability in patients with SIJD. However, the combination of these 2 therapies does not seem to bring about significantly better therapeutic results than either approach implemented separately<sup>29</sup>. A pilot study of Sacroiliac joint dysfunction treated with a single session of Stecco Method of Fascial Manipulation on 20 patients when applied at least 20 cm from the Posterior Inferior Iliac Spine was found to have decreased the pain around the SII and concluded that the inclusion of this type of approach in SIJD can allow for improved patient management, better tolerance for other treatments and a more rapid application of painfree exercise programs<sup>30</sup>. Another study on physiotherapy approaches in the treatment of sacroiliac joint dysfunction, a total of 64 patients with pain in the lumbosacral region due to SIJ dysfunction were treated. Divided in two group B (experimental n=41) and V (controlled n=23), the controlled group underwent treatment consisting of classic massage, core stability exercise on a stable surface, home exercise program auto-mobilization and for the experimental manipulative massage, manual mobilizations of SIJ, core stability exercise with a fitness ball. The study concluded that the positive influence of the therapy was common for both groups but more noticeable in the experimental. This method demonstrated the normalizing effect on the dysfunction of the pelvic girdle, reduction of muscle imbalance, decreased subjective complaints and symptoms of pain (VAS  $\pm 0.43$ , SEP  $\pm 0.23$  and LP  $\pm 0.70$ )<sup>22</sup>. A study undertaken to find Sacroiliac joint pain its anatomy, biomechanics, diagnosis, and treatment, suggested positive results stating that conservative treatment which included joint mobilization and Sacroiliac Joint belt was found to be effective<sup>23</sup>. Authors have also discussed a few current concepts in the treatment of Sacroiliac Joint Pain wherein, the importance and positive result of physical therapy combined with home-based exercises were found to be the mainstay. 12 And other approaches like intra-articular steroid injections provided a significant result in alleviating the pain but only for the short term. Simultaneously, the difficulty to completely combat the pain and disability through a specific treatment approach in a timeframe is observed even today. Therefore in search of better treatment protocols and methods for the management of SI joint pain, this study was carried out. This was a 12 months structured study aimed at determining the combined effects of piriformis stretch and muscle activating exercises (Group A) on patients with sacroiliac joint pain by measuring VAS For Pain and ODI for disability. The study also had a control group (Group B) who received a treatment protocol of muscle activation exercises. Each group consisted of 15 subjects who were randomly assigned and every single subject completed their therapy session. Therefore no drop-outs were recorded. The subjects were properly explained about the different treatment protocols and techniques that were included in the study and only after they have given a written consent were recruited in the study. A pre-treatment and a posttreatment assessment score of each outcome measure were

recorded and statistically analyzed. In this study, the experimental group (Group A) mean VAS score had decreased from 6.73 to 2.33, For ODI, it was seen that in group A, there was a significant difference between pre-test and post-test indicating that the protocol (the combined effect of piriformis stretch and muscles activation exercises) was effective, since the mean ODI score decreased from 40.30 to 16.13 An independent t-test was performed to compare the VAS scores of the patients with joint pain after treatment with piriformis stretch along with muscle activation exercises and patients with joint pain treated with muscle activation exercises. t = -2.714 which was statistically significant at a 5% probability level. It has been inferred that VAS was seen to have decreased significantly among patients treated with piriformis stretches along with muscle activation exercises as compared to patients treated with only muscle activation exercises implying that piriformis stretches along with muscle activation exercises was s more effective to reduce joint pain. An independent t-test was performed to compare the ODI scores of the patients with joint pain after treating with piriformis stretch along with muscle activation exercises and patients with joint pain treated with muscle activation exercises. t = -10.261 which was statistically significant at a 1% probability level. It has been inferred that ODI was seen to have decreased significantly among patients treated with piriformis stretches along with muscle activation exercises as compared to patients treated with only muscle activation exercises implying that piriformis stretches along with muscle activation exercises was more effective to reduce joint pain. Through this measure, we can state that the treatment protocol for group A had a significant improvement in pain and disability in patients with Sacroiliac Joint Pain. This study proved that piriformis stretch and muscle activation exercises were effective in the treatment of sacroiliac joint pain.

# 7 LIMITATIONS OF THE STUDY

This study consisted of a short course of treatment of 12 therapy sessions and the results showed only the short-term effects of the intervention and did not include long-term follow-up. The study was done with a very small sample size n=15.

# 8 ACKNOWLEDGEMENTS

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### 9 AUTHOR CONTRIBUTION STATEMENT

Dr. Abhijit Kalita (PT) and Dr. Bhupendra Karki (PT) conceptualized the study and the data. Dr. Bhupendra Karki (PT) carried out the study and gathered the data concerning this work. Dr. Abhijit Kalita (PT) and Dr. Abhijit Dutta analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology, results and contributed to the final manuscript.

All the authors read and approved the final version of the manuscript.

#### 10 CONFLICT OF INTEREST

Conflict of interest declared none.

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