

Time Difference Between Onsets of Lateral and Medial Hamstring Muscles During Gait in Patients With Patellofemoral Pain: A Preliminary Study

Dilber Karagozoglu Coskunsu^{1,2} , Filiz Can² , Shavkat Kuchimov³ , Nazif Ekin Akalan⁴ , I. Onder Kilicoglu⁵ , Necla Ozturk⁶ 

¹ Fenerbahçe University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Türkiye.

² Hacettepe University, Faculty of Physiotherapy and Rehabilitation, Istanbul, Türkiye.

³ Istanbul Kultur University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Türkiye.

⁴ Istanbul Kultur University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Türkiye.

⁵ Koc University, Faculty of Medicine, Department of Orthopaedic and Traumatology, Istanbul, Türkiye.

⁶ Maltepe University, Faculty of Medicine, Department of Biophysics, Istanbul, Türkiye.

Correspondence Author: Dilber Karagozoglu Coskunsu

E-mail: dilbercoskunsu@gmail.com

Received: 20.04.2021

Accepted: 06.02.2022

ABSTRACT

Objective: Early activation of lateral hamstrings (LH) relative to medial hamstrings (MH) has been thought to be the cause of abnormal knee abduction and external rotation of the tibia, which affects the orientation of patellar tendon and increases lateral patellofemoral compression. Therefore, early activation of LH relative to MH is considered to have a role in the patellofemoral pain (PFP). The aim of this study was to investigate the time difference between MH and LH onsets in patients with PFP during gait.

Methods: Thirteen patients with bilateral PFP (mean age 28.73±7.44 years) and 13 asymptomatic subjects (mean age 30.47±6.22 years) were recruited in the study. Gait analysis was performed using the ELITE system (BTS, Milano-Italy) with video cameras (TVC, BTS, Milano-Italy). Participants were requested to walk at a self-selected speed on a force platform, and EMG data were recorded from MH and LH muscles for 10 initial contacts by using TELEEMG (BTS, Milano-Italy). Time difference between the onsets of the MH and LH was calculated for each initial contact by using moving averaging method, then their mean was obtained for each participant.

Results: The time difference between onsets of MH and LH was – 26.9±22.2 ms for PFP subjects and – 11.2±14.2 ms for control subjects, and LH mainly became activated earlier compared to MH in most of the subjects in both groups. There was a statistically significant difference between the time differences of the groups ($p=0.041$).

Conclusion: Our findings suggest that LH displayed an earlier activation in subjects with PFP compared to control subjects during gait.

Keywords: Hamstrings, Surface EMG, Patellofemoral Pain, Gait., Time Difference

1. INTRODUCTION

Patellofemoral pain (PFP) is a common complaint experienced by physically active adults and adolescents (1), however its etiology has remained unclear (2). PFP is thought to be a result of abnormal patellar tracking and increased patellofemoral reaction forces that cause increased joint forces over the lateral patellar facets (1). Both altered knee and hip muscles activations were considered to be responsible for abnormal patellar tracking (2). Studies regarding activation timing on PFP have been mostly carried out on Vastus Medialis Obliques (VMO) and Vastus Lateralis (VL) muscles because of their crucial role in the patellofemoral kinematics (3). It was found that the relationship between timing of VMO and VL had changed in the patients with PFP (3). In addition, increased Gluteus Maximus activity (4) and delayed onset of the Gluteus Medius relative to the VMO during the weight bearing activities were reported (5,6). However, most of

the studies focused on the contribution of knee extensor muscles to PFP and the knee flexor group muscles received less attention (7-9).

Although hamstring muscles are primarily related to the tibiofemoral joint mechanics, they are considered to have effect on the patellofemoral joint as well. Experiments carried out on the effect of hamstring contractions on the patellar stability have provided evidence supporting the claim. It has been shown that contraction of the hamstring muscles causes posterior translation and external rotation of tibia, which affects the orientation of the patellar tendon (10-11). In an in vitro study, Elias et al. (2011) found that loading the hamstrings increased the patellar flexion, lateral tilt and lateral shift by 1°, 0.5° and 0.2°, respectively. Total contact force also increased at each flexion angle by 5% (12). Si-hyun et al. (13) measured activity of the medial and lateral

hamstring in standing position and suggested that dominant muscle contraction of lateral hamstring (LH), relative to medial hamstring (MH), may contribute to increased tibial rotation (13). As excessive tibial rotation is evident in patients with PFP clinically, this suggestion supports the clinical observations. In addition, it is generally agreed that an uneven onset of hamstrings can induce increased external tibial rotation with a preceded onset of LH in patients with PFP. To the authors' knowledge, there are only two published studies which directly investigated the relationship between the onsets of the LH and the MH in PFP (7,8). In one of these studies, the EMG activities of MH and LH hamstring muscles were assessed during voluntary isometric contraction in sitting position (7). According to the results of this study, the LH contracts earlier than the MH (7) compared to the control group. Dieter et al. (2014) investigated the muscle activation patterns of trained cyclists during cycling and they found significant difference between PFP and healthy groups in onset time of MH and LH muscles during cycling. Besides, they observed that LH activation occurred earlier than MH. Since PFP is a problem that aggravates mainly during dynamic activities, it is important to assess hamstring activity in dynamic activities. To this date, the study performed by Dieter et al. (2014) has been the only study investigating the activation onsets of MH and LH muscles during dynamic activity (8).

Therefore, we aim to investigate the time delay between the LH and MH in patients with PFP while they walk at a self-selected speed and compare them with healthy asymptomatic control subjects in order to elicit the role of hamstring muscles on the PFP. We hypothesized that patients with PFP would display greater time difference between MH and LH muscles, with the LH displaying an earlier activation than MH when compared to healthy control levels.

2. METHODS

The study was carried out at Gait Analysis Laboratory of Department of Orthopedics and Traumatology in University. This study was conducted in accordance with the rules of the Declaration of Helsinki. Written informed consent was obtained from each participant. The study was approved by the Ethics Committee of Hacettepe University (Approval number: GO14/646-31; Date:21.01.2015).

2.1. Subjects

Thirteen patients with bilateral PFP for the PFP group (8 females and 5 males; age: 30.46 ± 6.22 years, height: 167.87 ± 7.81 cm, weight: 67.87 ± 13.4 kg, BMI: 23.84 ± 2.84 kg/cm²) and 13 asymptomatic, healthy subjects for the control group (10 females and 3 males; age: 28.73 ± 7.43 years, height: 169.73 ± 7.09 cm, weight: 67.46 ± 14.31 kg, BMI: 23.15 ± 3.31 kg/cm²) were recruited in this study. As there was no available literature on the time delay between the onset of MH and LH during gait, we were unable to calculate a sample

size. We decided to conduct this preliminary study with 13 participants in each group.

Patients with PFP were screened to rule out other knee pathologies, and radiologic examination consisting of AP, lateral, and tangential radiograms was performed.

The patients who met the following criteria were included in the patient group: [1] bilateral pain arising from the patellofemoral articulation; [2] a pain intensity level of at least 3/10 point according to the Numeric Analog Scale (NAS) experienced while performing at least two of the following functional activities commonly associated with PFP: ascending or descending stairs, squatting, prolonged sitting, or kneeling, [3] a history of pain lasting longer than 2 months (14,15), [4] (4) being between 18-40 years of age and [5] body mass index <25.

The exclusion criteria were: [1] a history of knee surgery, [2] a history of patellar instability, or [3] neurological conditions that would affect gait (14).

All patients had bilateral complaints and the most severely affected leg was chosen for assessment. The participants in the control group had no knee pathology, existing knee pain or effusion that would affect their gait. The leg to be tested was chosen randomly for this group. None of the subjects in either group had played sports professionally at any point in their life. Demographic data, standing Q angle (16) and anthropometric measurements were recorded for all participants. Self-administered Kujala patellofemoral pain scale was used by PFP patients to determine their knee functions (17).

2.2. Experimental Set-up and Procedure

Silver/silver chloride, pre-gelled surface EMG electrodes (Ambu Blue Sensor, Denmark) were used to record the EMG signal while walking. Skin preparation and electrode placement were performed according to Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) criteria. The skin under the electrodes was cleaned by a skin preparation gel (NuPrep, Do Weaver and Co, USA) until the electrical impedance of the skin was less than 5 K Ω by using an impedance meter (Noraxon USA, INC, impedance meter) (). For the electrode placement, the participants were positioned prone on the table with their knee slightly flexed (less than 90°). The electrodes were placed at 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia while thigh in slight lateral rotation for LH (Biceps Femoris) muscles while they were placed at 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia while the thigh in slight lateral rotation for MH (Semitendinosus) muscle. Interelectrode distance was 20 mm between centers. A ground electrode was placed over the tuberositas tibia. Portable 8 channel TeleEMG (BTS, Milan, Italy) with built-in x10 amplifications, 10-450 Hz bandpass filter and 1000 Hz sampling rate were used to record EMG signals.

Six high-speed video cameras and 2 force platforms were synchronized with the surface EMG electrodes. The ELITE system (BTS, Milano, Italy) and video cameras (TVC, BTS, Milano, Italy) were used for gait analysis. Force platforms (Kistler Instruments, Winterthur, Switzerland) were buried in the middle of the walkway to record the ground reaction force, and detect the stance and swing phases used in the calculation of the onset of the initial contact (IC). Kinematic data were acquired and digitized with 100 Hz sampling rate.

Retroreflective spherical markers were placed according to Davis protocol over the bony landmarks to determine the joint centers and segment axis (Figure 1) (18). Next, the participants were invited to attend to a familiarization session to ensure their participation in the experiments since many wires would be placed on their bodies, which might result in discomfort while walking. The real recording sessions were initiated when the verbal start command was given. The participants were requested to walk at their self-selected speed on the 10 m long walkway. The procedure was repeated until the ICs of the evaluated side on the force platform were 10 records. The subjects were not informed of the existence of the force platform.

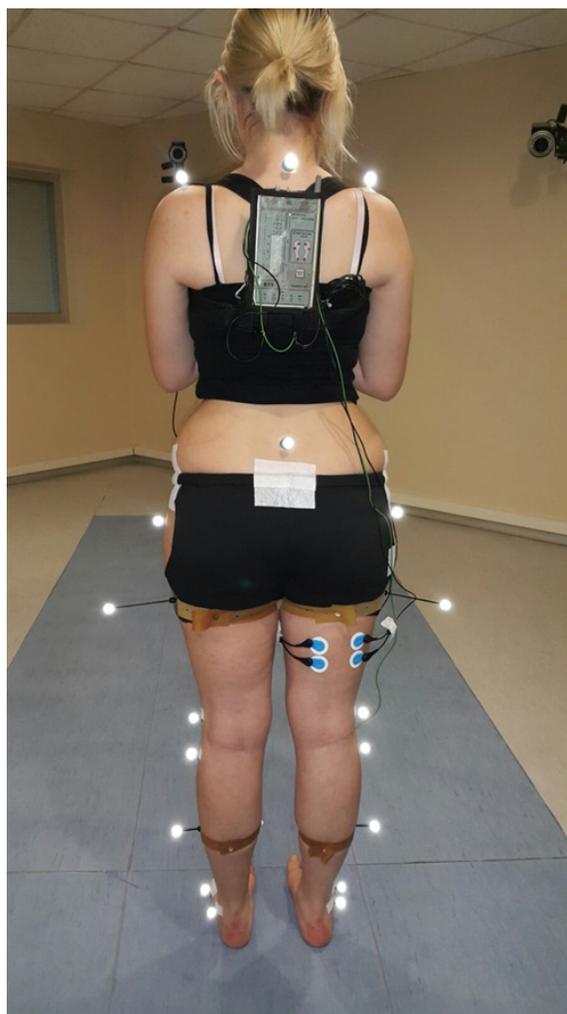


Figure 1. The participant ready for EMG recording

2.3. Data Processing

A custom prepared program written by MatLab (R2011) was used to detect the onsets of the LH and MH muscles. The raw EMG data for each epoch was full wave rectified and low pass filtered at 50 Hz (6th order Butterworth Filter). Then, it was averaged over a window of 20 ms, moving with a step of one sample. The onset of the EMG was identified as the point at which the signal deviated by more than three times the standard deviation of the baseline for a minimum of 25 ms (Figure 2) (19, 20). In addition, all data were also visually checked to ensure the onset time and there was no signal noise, which was possibly caused by movement artefacts (20). Then, the time delay between LH and MH was calculated as the difference between the onsets of EMG signals from LH and MH. A negative value meant that the onset of the LH occurred before the onset of the MH muscle. The mean of the time delays for 10 contractions was calculated for each participant.

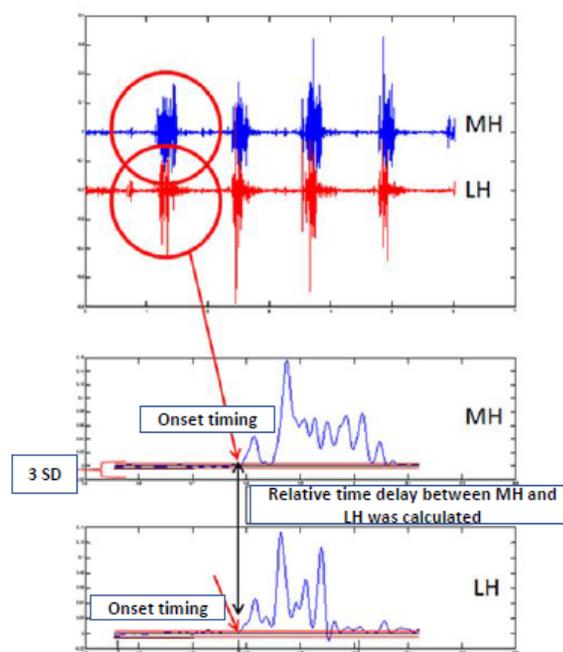


Figure 2. Calculation of the relative time delay between MH and LH. Calculation of the onset times for MH and LH muscle.

The following parameters were evaluated for the gait analysis: mean velocity (m/s), swing velocity (m/s) and kinematic evaluation of knee flexion angles at initial contact (IC) (°). Mean velocity (m/s) was assessed to ensure that any kinematic differences between groups were not due to differences in walking speed. Swing velocity was defined as the distance covered during the swing/swing duration and was assessed as hamstring's onsets were started just before initial contact. The knee flexion angle at IC (°) was evaluated as it might affect the onset of hamstring muscles.

2.4. Statistical Analysis

The data of this study were analyzed with the SPSS 20 for Windows (IBM, Armonk, New York). The normality was confirmed by Shapiro-Wilk testing with α set at 0.05. The independent sample-t test was used to determine the difference between the PFP and control groups. Statistical significance was set at $p < 0.05$.

3. RESULTS

There were no significant differences between the groups in age ($p=0.494$), height ($p=0.499$), weight ($p=0.938$) and BMI ($p=0.605$). There was no significant difference ($p=0.614$) in Q angle between the PFP ($15.13^\circ \pm 1.59^\circ$) and control group ($14.86^\circ \pm 1.24^\circ$). The mean Kujala patellofemoral pain score was found to be $81.2 (\pm 12.1)$ in the PFP group.

It was observed that onset of the activity of the MH and LH muscles prior to initial contact varied between individuals. In the control group, the LH muscles of 10 participants contracted earlier than the MH muscles, while the opposite result was found in the remaining 3 participants. In the PFP group, the LH muscles contracted before the MH muscles in 12 individuals, and the MH muscles contracted before the LH muscles in 1 individual (Table 1).

Table 1. Time difference between onsets of lateral and medial hamstring muscles. Time difference between LH and MH was computed by subtracting the onset time of MH from the onset time of LH (negative value means onset of LH started before the onset of the MH muscle)

PFP LH-MH (ms)	Control LH-MH (ms)
-19.8	-27.1
-26.5	-11.7
-34.5	-5.4
-33.8	-19.4
-16	-11.3
-23.7	15.9
20.1	-10.5
-69.1	-21.4
-12.2	2.2
-13.6	13.0
-22.6	-27.2
-37.4	-18
-60.7	-24.1

For the PFP group, the mean (\pm SD) time delay between the onsets of MH and LH was $-26.9 (\pm 22.2)$ ms whereas for the control group it was $-11.2 (\pm 14.2)$ ms. There was statistically significant difference in the time delays between the groups ($p=0.041$).

There were no statistically significant differences between the groups for mean velocity, swing velocity and IC knee flexion degree ($p=0.366$, 0.198 and 0.964 , respectively) (Table 2).

Table 2. Comparison of the mean velocity, swing velocity and IC knee flexion degrees of the control and PFP groups.

	Control (n=15) mean \pm SD	PFP (n=15) mean \pm SD	p value
Mean Velocity (m/s)	1.12 \pm 0.10	1.17 \pm 0.13	0.366
Swing Velocity (m/s)	2.79 \pm 0.21	2.92 \pm 0.29	0.198
IC knee flexion ($^\circ$)	3.898 \pm 4.86	3.942 \pm 5.46	0.964

4. DISCUSSION

This case-control study reveals that there was statistically significant time delay difference between the activation onsets of the LH and MH in patients with PFP when compared to healthy subjects during gait.

While walking, the long head of the biceps femoris is activated in the last part of the swing phase, aiming to decelerate both hip flexion and knee extension, and it also prepares the lower limb for the IC, resulting in load absorption. In IC, plantar flexion occurs, controlled by eccentric contraction of tibialis anterior and increased flexion of the knee joint, followed by knee extension (21). At that stage during IC, the knee absorbs load and shows symptoms in case of any pathology caused either by an internal derangement or imbalance of the musculoskeletal dynamics. That is why we defined the IC as a reference point in time in our study and investigated which part of the hamstrings (medial or lateral) was activated first before this reference point. If LH or MH displays an altered onset time, it is expected to observe altered rotational moments affecting the knee kinematics and lead to the maltracking of the patella.

There is limited number of studies in the literature which primarily focused on investigating timing of hamstring muscles in PFP in comparison to asymptomatic subjects. In Patil et al.'s (2011) study, the subjects were asked to perform maximal voluntary isometric contraction while sitting on an adjustable chair with their hip at 90° flexion and their knee at 45° flexion. While the time delay between onset times of LH and MH was found as 62 ± 73.1 ms in the control group, it was found 8.2 ± 80.9 ms in the PFP group and, the results revealed that LH was activated earlier than MH (7). However, when subject is sitting, testing the hamstrings will reveal results about selective activation of these muscles since the hip is relatively well stabilized and all moments from other parts of the body are ignored. Since PFP is a problem that is aggravated mainly during dynamic activities, it is important to assess hamstring activation in dynamic activities.

In another study, Dieter et al. (8) investigated the muscle activation patterns of LH and MH muscles in cyclists with and without PFP. The results of this study showed that cyclists with PFP exhibited altered temporal characteristics in their muscle activation patterns compared to those without PFP. The average onset of LH muscle occurred 39 ± 44 ms after MH muscle onset in control group, whereas the LH onset occurred 111 ± 78 ms before MH onset in the PFP group.

According to our results, in both PFP and control groups, LH was activated earlier than MH: for the PFP group, LH was activated 26.9 (± 22.2) ms earlier while in the control group, LH was activated 11.2 (± 14.2) ms earlier than MH. However, the time difference between the onsets of LH and MH was statistically significant. Therefore, our results support previous studies (7,8).

One of the parameters which may affect EMG data and kinematic parameters is speed of walking. In our study, the participants were asked to walk at their self-selected speed since walking at comfortable speed improves repeatability of EMG data (2). In their systematic review, Barton et al. (2009) (22) concluded that velocity is one of the temporospatial gait characteristics which shows trend towards reduction in PFP patients, and only in one study, significant reductions in knee flexion angle were found at IC in the sagittal plane (23). In our study, there were no statistically significant differences between the mean velocity, swing velocity of the gait and IC knee flexion angles of the groups.

Another parameter which may affect the results is the symptom severity. According to Crossley et al. (2004) (24), a Kujala score of 70 implies moderate pain and disability. In our study, the mean Kujala score of the PFP subjects was 81.2 which is higher than the threshold that Crossley et al. (2004) (24) stated. As the higher score means more disability, the time difference between the onsets could be higher if the participants with PFP were more severely affected.

To our knowledge, this is the first study that assessed the time difference between the onsets of MH and LH muscles during gait. However, this study has some limitations that should be highlighted. We could not perform priori sample size analysis as there was no data to use for calculation. To inform future studies, we performed post-hoc sample size estimation to find out how many participants would be required to adequately conduct a future study to detect differences between the groups in medial–lateral hamstring timing. This calculation was carried out with a significance level of 0.05, 80% power, and using the mean and standard deviations of the groups. The calculation showed an estimated sample size of 22 for each group. Further research with a larger sample size is therefore recommended.

5. CONCLUSION

This study was conducted to find out whether the time delay between the activation onsets of the MH and the LH differs in patients with PFP when compared to healthy subjects. Although there were large variations between the subjects, significant difference was found in the time delays between the groups. The results showed that the time difference between the onsets of MH and LH changed in PFP patients compared to the healthy participants and LH displayed an earlier activation than MH.

Acknowledgment: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The first author would like to thank Prof. Dr.

Kaya Akan, Prof. Dr. Kerem Birsell, and Dr. Haris Begovic for their contribution to this study, and Lecturer Yasar Erdin for his efforts in checking the language quality of the final draft.

Conflict of interest: There is no conflict of interest for this study.

REFERENCES

- [1] Powers CM, Witvrouw E, Davis IS. Evidence-based framework for a pathomechanical model of patellofemoral pain: 2017 patellofemoral pain consensus statement from the 4th International Patellofemoral Pain Research Retreat, Manchester, UK: part 3. *Br J Sports Med* 2017; 51:1713–1723.
- [2] Bolgla, L. A., Malone, T. R., Umberger, B. R., Uhl, T. L. Comparison of hip and knee strength and neuromuscular activity in subjects with and without patellofemoral pain syndrome. *International journal of sports physical therapy*, 2011; 6(4):285.
- [3] Chester, R., Smith, T. O., Sweeting, D., Dixon, J., Wood, S., Song, F. The relative timing of VMO and VL in the aetiology of anterior knee pain: a systematic review and meta-analysis. *BMC musculoskeletal disorders*, 2008; 9(1):64.
- [4] Souza RB, Powers CM. Differences in hip kinematics, muscle strength, and muscle activation between subjects with and without patellofemoral pain. *J Orthop Sports Phys Ther*. 2009;39(1):12-19.
- [5] Brindle TJ, Mattacola CG, McCrory JL. Electromyographic changes in the gluteus medius during stair ascent and descent in subjects with anterior knee pain. *Knee Surg Sports Traumatol Arthrosc*. 2003; 11:244-251.
- [6] Cowan SM, Crossley KM, Bennell KL. Altered hip and trunk muscle function in individuals with patellofemoral pain syndrome. *Br J Sports Med*. 2009; 43:584-588.
- [7] Patil S, Dixon J, White LC, Jones AP, Hui AC. An electromyographic exploratory study comparing the difference in the onset of hamstring and quadriceps contraction in patients with anterior knee pain. *Knee* 2011; 18(5):329-332.
- [8] Dieter BP, McGowan CP, Stoll SK, Vella CA. Muscle activation patterns and patellofemoral pain in cyclists. *Medicine & Science in Sports & Exercise*. 2014 Apr 1;46(4):753-756.
- [9] Liebensteiner M.C., Szubski C., Raschner C., Krismer M., Burtscher M., Platzer HP, Deibl M., Dirnberger E. Frontal plane leg alignment and muscular activity during maximum eccentric contractions in individuals with and without patellofemoral pain syndrome. *Knee* 2008; 15: 180–186.
- [10] Li G., DeFrate L.E., Zayontz S., Park S.E., Gill T.J. The effect of tibiofemoral joint kinematics on patellofemoral contact pressures under simulated muscle loads *Journal of Orthopaedic Research* 2004; 22 (4): 801-806.
- [11] Kumagai M, Mizuno Y, Mattessich SM, Elias JJ, Cosgarea AJ, Chao AJ. Posterior Cruciate Ligament Rupture Alters In Vitro Knee Kinematics *Clin Orthop Relat Res* 2002; 395:241-248.
- [12] Elias JJ, Kirkpatrick MS, Saranathan A, Mani S, Smith LG, Tanaka MJ. Hamstrings loading contributes to lateral patellofemoral malalignment and elevated cartilage pressures: an in vitro study. *Clin Biomech (Bristol, Avon)* 2011; 26(8):841-846.
- [13] Si-hyun Kim, Oh-yun Kwon, Kyue-nam Park, Sung-dae Choung. Correlation between the Angle of Lateral Tibial Rotation and the Ratio of Medial and Lateral Hamstring Muscle Activities

- during Standing Knee Flexion *J. Phys. Ther. Sci.* 2013; 25 (4): 383-386.
- [14] Powers CM, Ward SR, Chan LD, Chen YJ, Terk MR. The effect of bracing on patella alignment and patellofemoral joint contact area *Med Sci Sports Exerc.* 2004; 36:1226-1232.
- [15] Souza, R.B. Powers C.M. Predictors of Hip Internal Rotation During Running – An Evaluation of Hip Strength and Femoral Structure in Women with and without Patellofemoral Pain *Am J Sports Med* 2009; 37(3): 579-587.
- [16] Woodland L.H., Francis R.S., Parameters and comparisons of the quadriceps angle of college-aged men and women in the supine and standing positions *Am J Sports Med* 1992; 20(2):208-211.
- [17] Kuru T, Dereli EE, Yaliman A, Validity of the Turkish version of the Kujala patellofemoral score in patellofemoral pain syndrome *Acta Orthop Traumatol Turc* 2010; 44(2),152-156.
- [18] Davis R, Ounpuu S, Tyburski D, Gage J. A gait analysis data collection and reduction technique. *Hum Movement Sci* 1991; 10:575 – 587.
- [19] Cowan S.M, Bennell K.L., Hodges P.W, Mc Connel J. The test-retest reliability of the onset of concentric and eccentric vastus medialis obliquus and vastus lateralis electromyographic activity in a stair stepping task. *Physical Therapy in Sport* 2010; 1: 129-136.
- [20] Hodges P W, Bui B H 1996 A comparison of computer-based methods for the determination of onset of muscle contraction using electromyography *Electroencephalography and Clinical Neurophysiology* 1996; 101: 511-519.
- [21] Stewart C, Jonkers I, Roberts A. Estimation of hamstring length at initial contact based on kinematic gait data. *Gait Posture.* 2004; 20(1):61-6.
- [22] Barton CJ, Levinger P, Menz HB, Webster K. Kinematic gait characteristics associated with patellofemoral pain syndrome: a systematic review *Gait&Posture* 2009; 30(4): 405-416.
- [23] Powers CM, Heino JG, Rao S, Perry J. The influence of patellofemoral pain on lower limb loading during gait. *Clin Biomech* 1999; 14:722–728.
- [24] Crossley K, Bennell K.L., Cowan S., Green S., Analysis of Outcome Measures for Persons with Patellofemoral Pain: Which are Reliable and Valid? *Arch Physical Medicine and Rehabilitation* 2004; 85:815-822 .

How to cite this article: Karagozoglu Coskunsu D, Can F, Kuchimov S, Akalan NE, Kilicoglu IO, Ozturk N. Time Difference Between Onsets of Lateral and Medial Hamstring Muscles During Gait in Patients With Patellofemoral Pain: A Preliminary Study. *Clin Exp Health Sci* 2022; 12: 192-197. DOI: 10.33808/clinexphealthsci.923264