

Muscular activity differences and mechanisms for backhand straight and backhand cross in squash

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ABSTRACT

The purpose of the current study was to identify the differences of the upper extremity muscle activations around three joints of the dominant arm during two patterns of backhand strokes. Ten elite female right-handed squash players participated (age: 18.4 ± 0.8 years; mass: 60.8 ± 1.8 kg; height: 165.2 ± 1.6 cm). EMG data from six muscles around the shoulder, elbow, and wrist joints were recorded. The AD muscle activity of the backhand straight was greater throughout the execution and follow-through phases than the backhand cross (p < .001). In contrast, muscular activity in the PD muscle was greater during the three phases of backhand cross than backhand straight (p < .001). Elbow muscles showed no significant differences except in the follow-through phase, where the TB muscle demonstrated increased activity in the backhand straight. The WF and WE muscles had similar patterns in both strokes. This study provides novel insights into arm muscle activation during two patterns of backhand stroke in squash. Understanding the muscle activity mechanisms of these patterns can inform training strategies, optimize performance, and prevent injury risks to the shoulder, elbow, and wrist during the phases of the two patterns of backhand stroke in squash.

Keywords: Biomechanics, Wearable, Electromyography, Physical activity, Backhand cross, Backhand straight.

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INTRODUCTION

Muscular activity is essential in the game of squash due to its fast-paced nature, dynamic motions, and forceful strokes. Players must possess exceptional muscular strength, endurance, and control. Consequently, muscle activity plays a crucial role in squash. It helps with strength, speed, endurance, injury avoidance, and movement efficiency. Players may improve their performance, reduce their risk of injury, and eventually raise their game on the squash court by working on physical strength, endurance, and control (Akl, Hassan, Elgizawy, Tilp, & health, 2021; Karess et al., 1991; Kim, Min, Subramaniyam, & Kim; Locke et al., 1997; Tapie, Gil, Thoreux, & Making, 2020).

Therefore, the interaction and complementarity of motor abilities and needs in terms such as physiological, kinetics, and cognition variables are crucial for performance success (Finch & Eime, 2001; Horobeanu, Johnson, & Pullinger, 2019; Lees, 2002; Mohammed, 2015; Okhovatian & Ezatolahi, 2009).

However, scientific research into the acquisition of EMG data to record the activity of muscles, particularly in squash strokes, is quite required (Safikhani, Kamalden, Amri, & Ahmad, 2015). To date, no research has exclusively focused on comparing the kinetic, kinematic, and muscular activity of backhand cross and backhand straight in squash, simultaneously (Akl, Hassan, Elgizawy, & Tilp, 2021; Cho & Kim, 2007). Specifically, there's a gap in understanding the movement and muscle activity in the upper limb during forehand and backhand stroke patterns in squash (Akl, Hassan, Elgizawy, & Tilp, 2021; Yaghoubi, Moghadam, Khalilzadeh, & Shultz, 2014).

The frequency of backhand strokes (63.1 percent) surpassing forehand strokes (36.9 percent) highlights the importance of the backhand stroke in squash, Nevertheless, there are noticeable differences in the abilities utilized throughout a squash game (Hong, Chang, & Chan, 1996). Previous studies have investigated the relationship between racket speed and upper extremity circular motion (Elliott, Marshall, & Noffal, 1996), kinematic analysis of forehand stroke (Hong et al., 1996), and the upper extremity segment's kinematics during backhand strokes (An, Ryu, Ryu, Soo, & Lim, 2007).

The backhand cross is an important stroke in squash because it allows players to change the direction of the rally and put pressure on their opponent. With its ability to create angles and open up the court, the goal is to hit the ball diagonally across the court, aiming for the opposing front corner, which is often the opponent's weaker side (Fernández Pérez, García Hernández, & Díaz Miranda, 2020; Ghani, Zainuddin, & Ibrahim, 2018). In many game situations, the backhand straight stroke may be employed tactically.

Offensively, it enables players to exert pressure by hitting the ball with speed and precision, forcing the opponent to respond fast and perhaps opening up for further strokes. Defensively, it may be used to reset the rally and retake control by returning the ball deep and near to the side wall, limiting the attacking possibilities of the opponent (Carboch, Dušek, & Sport, 2023; Ghani, 2013; Pérez, Hernández, & Miranda, 2020).

To summarize, the backhand cross is a fundamental squash stroke that provides players with a vital option for shifting the direction of the rally and applying pressure to their opponents (Fernández Pérez et al., 2020; Ghani et al., 2018). The backhand straight stroke, another fundamental skill, enables players to strike the ball with power and precision along the side wall (Carboch et al., 2023; Ghani, 2013; Pérez et al., 2020). Additionally, incorporating specific drills and workouts that promote muscular coordination, timing, and

sequencing. This involves a focus on controlled motions to develop improved technique during various squash-related activities (Girard, Micallef, Noual, & Millet, 2010; Masu & Otsuka, 2021).

Thus, understanding the nature of backhand pattern performance, the differences between the two patterns, and the relationship between training volume or intensity and the types and grades of injuries helps coaches and players acquire the proper technique and practice, addressing coordination issues and optimizing performance, and players can effectively use this stroke to gain an advantage on the court and improve their overall game (Akyol, 2012; Drew & Finch, 2016; Leeder, Horsley, & Leeder, 2016; Vanrenterghem, Nedergaard, Robinson, & Drust, 2017).

Therefore, it appears critical to research the mechanism of muscle activation during backhand stroke patterns. This study may provide further information regarding the significance of muscle activation in squash for joint support and stability, maintaining optimal body alignment, absorbing impact pressures, and reducing the risk of injury (Pallis, McNitt-Gray, & Hung, 2019).

Surprisingly, no study has yet explored the differences and processes of muscle activation during both of the backhand strokes (backhand cross and backhand straight). Therefore, the purpose of the current study is to identify the differences of the upper extremity muscle activations around three joints of the dominant arm during two patterns of backhand strokes. We hypothesized that muscular activation in the upper extremity muscles would differ across the two backhand stroke patterns as well as within each pattern's backhand stroke phases, particularly during the execution phase.

MATERIALS AND METHODS

Subjects and study design

We recruited ten healthy volunteers, all elite female squash players (age: 18.4 ± 0.8 years; mass: 60.8 ± 1.8 kg; height: 165.2 ± 1.6 cm). The subjects participated in professional squash tournaments and had official Egyptian squash federation rankings ranging from 4 to 20 (national and international). The study was authorized by the institution's studies and research ethics committee after the subjects gave written informed consent.

We utilized a cross-sectional design with repeated measures, in which all participants conducted three successful trials while recording EMG measurements for electrical muscle activity. of six upper extremity muscles: anterior deltoid (AD), posterior deltoid (PD), biceps brachii (BB), triceps brachii (TB), wrist flexor (WF), and wrist extensor (WE).

Experiment protocol

Following a 15-minute warm-up that comprised general, elbow, and shoulder mobility exercises, stretching, and protocol familiarization, participants performed squash backhand straight and crosscourt strokes. Each participant provided multiple successful tries, with a one-minute break in between. The backhand cross and straight squash abilities were subdivided into three phases: preparation, execution, and follow-through. From the start of the movement until the end of the elbow flexion, the preparation phase was defined; from the start of the shot, the execution phase was defined; and from the moment of the shot to the conclusion of the movement, the follow-through phase was defined, see Figure 1. Video analysis employing 3D simi motion capture, synchronized with EMG, characterized the phases.



Figure 1. Backhand stroke phases (preparation phase, execution phase, follow-through phase).

sEMG activity recording and analysis

Surface EMG (Myon m320RX; Myon, Switzerland) was used to record the selected muscles of the dominant arm. The upper extremity muscles' skin was shaved and cleaned with alcohol before bipolar, circular 10 mm diameter silver chloride surface electrodes (SKINTACT FS-RG1/10, Leonhard Lang GmbH, Austria) were placed to the chosen muscles (Figure 2). Following the SENIAM recommendations, electrodes were placed over each muscle (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). The EMG signals were sampled at 1000 Hz and processed using a 16-bit analogue-to-digital (A/D) converter.



Figure 2. Electrode placements of the selected muscles (Black dots).

Visual 3D software was used to analyse EMG data (C-Motion, MD, USA). A Butterworth filter was used to band-pass filter raw EMG data (20 Hz-450 Hz). The signals were pre-processed using a full-wave rectifier and a linear envelope created using the root mean square (RMS) technique with a window size of 100 ms. After each individual completed the experimental activities, the data were normalized to an isometric maximal voluntary contraction (MVC). To get the MVC values, subjects performed three 5 s repeats with a 60 s rest interval while sitting in a comfortable and stable chair with forearm resistance. The MVC value was calculated by averaging peak muscular activity across three repetitions for each muscle.

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Statistical analysis

Means and confidence intervals (mean CI) were used to report descriptive statistics. The Shapiro-Wilk test was utilized to investigate the normality of the data, and all data were found to be appropriate for parametric analysis. A paired T-test and Gardner-Altman estimate plots were used to identify significant differences and compare the mean of each variable across the two backhand stroke patterns (Cross and Straight) in squash. The IBM SPSS Statistics v21 software was used for statistical analysis.

RESULTS

EMG raw data (a), EMG RMS (b), and average values, coefficient interval, and paired T-test for the normalized EMG (%MVC) are presented for the preparation (c), execution (e), and follow-through (g) phases. The Gardner-Altman estimation plots showing the paired mean differences between the cross and the straight for Squash backhand stroke related to muscle activity present the effect size as a confidence interval (95% CI) on separate but aligned axes, where the confidence interval of the mean difference is displayed to the right of the data during the three analysed of backhand phases.



Note. (a) raw data, and (b) RMS data. Average values and coefficient interval for the normalized EMG (%MVC) and Gardner-Altman estimation plot of AD per skill of the muscles during the three phases of performance (c, d; e, f; and g, h, respectively.

Figure 3. Backhand Cross and Backhand Straight muscle activity of AD muscle activity.

Figure 3 shows the average values and coefficient intervals for the AD muscle. The paired T-test demonstrated significant main effects for backhand strokes for AD muscle activity during the preparation (Figure 3c), execution (Figure 3e), and follow-through phase (Figure 3g). During preparation, there was no significant difference in AD muscle activity during the backhand cross compared to the backhand straight (p = .783, Figure 3c). However, there was a significant difference during execution (p < .001, Figure 3e) and follow-through (p < .001, Figure 3g). And the estimation plots demonstrated the differences between backhand cross and backhand straight during the three analysed phases: the preparation = 0.263 (Figure 3d), the execution = 0.541 (Figure 3f), and the follow-through phase = -0.4 (Figure 3h).



Note. (a) raw data, and (b) RMS data. Average values and coefficient interval for the normalized EMG (%MVC) and Gardner-Altman estimation plot of PD per skill of the muscles during the three phases of performance (c, d; e, f; and g, h, respectively.

Figure 4. Backhand Cross and Backhand Straight muscle activity of PD muscle activity.

Figure 4 shows the average values and coefficient intervals for the PD muscle. The paired T-test demonstrated significant main effects for backhand strokes for PD muscle activity during the preparation (Figure 4c), execution (Figure 4e), and follow-through phase (Figure 4g). There was a significant difference between PD muscle activity during the backhand cross compared to that present during the backhand straight

during the three analysed phases: the preparation (p < .001, Figure 4c), the execution (p < .001, Figure 4e), and the follow-through phase (p < .001, Figure 4g). And the estimation plots demonstrated the differences between backhand cross and backhand straight during the three analysed phases: the preparation = -6.907 (Figure 4d), the execution = -20.211 (Figure 4f), and the follow-through phase = -13.646 (Figure 4h).



Note. (a) raw data, and (b) RMS data. Average values and coefficient interval for the normalized EMG (%MVC) and Gardner-Altman estimation plot of BB per skill of the muscles during the three phases of performance (c, d; e, f; and g, h, respectively.

Figure 5. Backhand Cross and Backhand Straight muscle activity of BB muscle activity.

Figure 5 shows the average values and coefficient intervals for the BB muscle. The paired T-test demonstrated significant main effects for backhand strokes for BB muscle activity during the preparation (Figure 5c), execution (Figure 5e), and follow-through phase (Figure 5g). There was a non-significant difference between BB muscle activity during the backhand cross compared to that present during the backhand straight during the three analysed phases: the preparation (p = .434, Figure 5c), the execution (p = .432, Figure 5e), and the follow-through phase (p = .874, Figure 5g). And the estimation plots demonstrated the differences between backhand cross and backhand straight during the three analysed phases: the



preparation = -0.957 (Figure 5d), the execution = -0.829 (Figure 5f), and the follow-through phase = 0.263 (Figure 5h).

Note. (a) raw data, and (b) RMS data. Average values and coefficient interval for the normalized EMG (%MVC) and Gardner-Altman estimation plot of TB per skill of the muscles during the three phases of performance (c, d; e, f; and g, h, respectively.

Figure 6. Backhand Cross and Backhand Straight muscle activity of TB muscle activity.

Figure 6 shows the average values and coefficient intervals for the TB muscle. The paired T-test demonstrated significant main effects for backhand strokes for TB muscle activity during the preparation (Figure 6c), execution (Figure 6e), and follow-through phase (Figure 6g). There was a non-significant difference between TB muscle activity during the backhand cross compared to that present during the backhand straight during the preparation phase (p = .223, Figure 6c) and the execution phase (p = .532, Figure 6e). And a significant difference was observed during the follow-through phase (p < .05, Figure 6g). And the estimation plots demonstrated the differences between backhand cross and backhand straight during the three analysed phases: the preparation = 1.00 (Figure 6d), the execution = -0.939 (Figure 6f), and the follow-through phase = 3.144 (Figure 6h).





Figure 7. Backhand Cross and Backhand Straight muscle activity of WF muscle activity.

Figure 7 shows the average values and coefficient intervals for the WF muscle. The paired T-test demonstrated significant main effects for backhand strokes for WF muscle activity during the preparation (Figure 7c), execution (Figure 7e), and follow-through phase (Figure 7g). There was a non-significant difference between WF muscle activity during the backhand cross compared to that present during the backhand straight during the three analysed phases: the preparation (p = .069, Figure 7c), the execution (p = .625, Figure 7e), and the follow-through phase (p = .534, Figure 7g). And the estimation plots demonstrated the differences between backhand cross and backhand straight during the three analysed phases: the preparation = 1.386 (Figure 7d), the execution = 0.865 (Figure 7f), and the follow-through phase = 0.633 (Figure 7h).

Figure 8 shows the average values and coefficient intervals for the WE muscle. The paired T-test demonstrated significant main effects for backhand strokes for WE muscle activity during the preparation (Figure 8c), execution (Figure 8e), and follow-through phase (Figure 8g). There was a non-significant

difference between WE muscle activity during the backhand cross compared to that present during the backhand straight during the three analysed phases: the preparation (p = .486, Figure 8c), the execution (p = .215, Figure 8e), and the follow-through phase (p = .808, Figure 8g). And the estimation plots demonstrated the differences between backhand cross and backhand straight during the three analysed phases: the preparation = 0.543 (Figure 8d), the execution = 1.564 (Figure 8f), and the follow-through phase = -0.400 (Figure 8h).



Note. (a) raw data, and (b) RMS data. Average values and coefficient interval for the normalized EMG (%MVC) and Gardner-Altman estimation plot of WE per skill of the muscles during the three phases of performance (c, d; e, f; and g, h, respectively.

Figure 8. Backhand Cross and Backhand Straight muscle activity of WE muscle activity.

DISCUSSION

The purpose of the current study was to explore the arm muscle activations regarding performance and injury prevention during different patterns of backhand stroke in squash. To our knowledge, this is the first paper to demonstrate the importance of determining the muscle activity during the cross and straight squash backhand strokes using electromyographic analysis, which may help to increase the information about the

muscular mechanisms of each pattern of backhand stroke, focus on the most appropriate exercises and training methods, and improve the quality of performance to prevent injury.

The muscle activity of AD, PD, BB, TB, WF, and WE were examined during the three phases of performance, when they activated as prime movers' muscles of the arm during backhand strokes in squash. The muscles being evaluated work around three upper-extremity joints. They control adduction and abduction at the shoulder, flexion and extension at the elbow, and radial deviation at the wrist.

Shoulder muscles

The muscles around the shoulder play a different role during backhand cross than in backhand straight strokes. During both backhand strokes, we can assess muscle activity based on its function and the role of muscle in performance. At the shoulder, AD muscle showed high activity of the backhand cross and backhand straight during the preparation phase, then decreased during the execution phase and increased again during the follow-through phase, as it provided power by abducting the shoulder. Its action appeared to be a deceleration of the backhand cross during the execution phase. In context, the action continues to keep the level of activity of the backhand straight during the execution phase, and then both patterns of strokes increase during the follow-through phase (Morris, Jobe, Perry, Pink, & Healy, 1989). Overall, the AD muscle is more active during backhand straight than backhand cross. There were no significant differences observed in the amplitude of the AD muscle activation during the preparation phase of the backhand cross and backhand straight. Subsequently, the AD muscle demonstrated significant differences in muscle activity during the execution phase and follow-throw phase of the backhand cross and backhand straight strokes, this finding is in agreement with Yaghoubi et al. (2014) (Yaghoubi et al., 2014).

In addition, the PD muscle showed high activity of the backhand cross during the execution phase and the follow-through phase, with less activity during the preparation phase. Nevertheless, the PD muscle activity is higher on the backhand cross than the backhand straight during the three phases of performance. In context, the PD muscle activity increased during the backhand straight, from the preparation phase to the followthrough phase. In contrast, the PD muscle is more active during the backhand cross than the backhand straight when compared to the AD muscle activity. This result is clearly demonstrated by the high significant differences in the amplitude of the PD muscle activation between both patterns of the backhand strokes (backhand cross and backhand straight) during the three phases of performance, and it is supported by the Gardner-Altman estimation plot method. During backhand strokes, the shoulder and trunk rotate, and as the body weight shifts, energy is transmitted to the dominant arm, requiring considerable horizontal abduction to place the upper limb posterior to the trunk (Morris et al., 1989; Yaghoubi et al., 2014). Thus, the AD and PD muscles act to stabilize the arm as a rigid extension of the racket by interactions, as they are the agonist and antagonist muscles around the shoulder during both patterns of the backhand strokes, as both utilize a forward action of the upper extremity (Yaghoubi et al., 2014). The backhand strokes required greater activity of the PD to maintain the abducted shoulder position necessary in the backhand performance, this finding agrees with Yaghoubi et al. (2014) (Yaghoubi et al., 2014).

Elbow muscles

Two of the muscles examined (BB and TB) work on the elbow hinge. The BB and TB are only responsible for controlling elbow flexion and extension. The BB showed lower activity during the backhand strokes, and in addition to performing elbow flexion, they also controlled forearm rotation. This means that the muscles have a positioning role, and that the elbow joint's inherent stability maintains position against the forces of both patterns of backhand strokes and a flexed position during the preparation phase. Except for an increase in activity during the execution and follow-through phases, the BB muscle showed reduced activity during the

preparation phase of both patterns of backhand strokes, resulting in prolonged durations of the preparation phase (Akl, Hassan, Elgizawy, & Tilp, 2021; Yaghoubi et al., 2014). This means that, in addition to putting the elbow in flexion, the BB muscle was stabilizing the elbow flexion and contributing to the forearm's stability against rotation (Akl, Hassan, Elgizawy, & Tilp, 2021; Morris et al., 1989). The BB muscle showed no significant differences in the muscle activation between both patterns of the backhand strokes (backhand cross and backhand straight) during the three phases of performance. At the elbow, the TB muscle showed high activity only during the execution phase and the early of the follow-through phase, as they provided power by extending the elbow (Akl, Hassan, Elgizawy, & Tilp, 2021). At the forearm, again, the BB and TB muscles showed an increase in activity in the early of the follow-through phase, and in the late of the followthrough phase, the TB increased during the backhand straight rather than backhand cross. Because both the backhand straight and the backhand cross require the elbow to extend across a wide range of motion and against resistance, the TB is engaged for lengthier periods of time throughout both patterns of backhand strokes, particularly during the execution phase. This result was clearly demonstrated by the moderately significant differences in the amplitude of the TB muscle activation between both patterns of the backhand strokes (backhand cross and backhand straight) during the follow-through phase (p = .014), and it is supported by the Gardner-Altman estimation plot method. Furthermore, no significant variations in BB and TB amplitude were identified throughout other phases of performance. This finding is in agreement with previous research in other activities (Werner, Fleisig, Dillman, & Andrews, 1993; Yaghoubi et al., 2014). Backhand strokes generate substantial trunk rotation and horizontal abduction of the shoulder, resulting in a strong centrifugal force at the elbow joint. Because the forces generated at the proximal joint were higher than those produced at the elbow (Sisto, Jobe, Moynes, & Antonelli, 1987; Yaghoubi et al., 2014), both the BB and TB muscles performed a similar role of transmitting energy during the backhand strokes. Thus, no differences in the BB and TB muscles amplitude were found between both patterns of the backhand strokes (backhand cross and backhand straight) (Yaghoubi et al., 2014).

Wrist muscles

The wrist was the third joint involved in muscular activity. Flexion and extension motions demonstrate wrist and grip stability. During both patterns of backhand strokes, the WF and WE were examined. They worked cooperatively to support the wrist during both patterns. The WF muscle was highly active during both patterns when flexor moment resistance was necessary due to execution and ball contact (Morris et al., 1989). While hitting the single-handed backhand stroke, the WE muscle activity increased as the impact time approaches, which is likely necessary for wrist function throughout the racket swing (Furuya et al., 2021; Charles E. Giangarra, Betty Conroy, Frank W. Jobe, Marilyn Pink, & Jacquelin Perry, 1993). This is most likely because the WF and WE muscles act as stabilizers during forehand and backhand strokes, respectively (Furuya et al., 2021).

The amplitude of the WF was higher during the execution phase at the most distal joint because the ball is cupped between the forearm and hand during the backhand stroke, requiring a greater amplitude of the WF muscle (Yaghoubi et al., 2014). This is most likely because both the backhand cross and backhand straight rely on a pushing movement incorporating elbow extension. Thus, for the backhand strokes, the WE muscle activity was higher during the execution and follow-through phases during both patterns as compared to the WF muscle activity, which was higher during the execution phase only. This finding is in agreement with Furuya et al. (2021) (Furuya et al., 2021). Furthermore, WE muscle activation was common throughout both backhand stroke patterns. A previous study of EMG activity during tennis strokes showed a similar trend. Clearly, the high level of activity in the WE muscle during the backhand stroke helps to keep grip stability in an extension and radial deviation wrist position throughout execution and early follow-through. This is interesting because the wrist extensors are the most commonly injured muscle among racket players

(Charles E Giangarra, Betty Conroy, Frank W Jobe, Marilyn Pink, & Jacquelin Perry, 1993). The WF and WE muscles showed no significant changes between the two backhand stroke patterns. Thus, this finding demonstrates that these muscles had a comparable function in terms of muscular and temporal activity in the backhand cross and backhand straight strokes (Yaghoubi et al., 2014).

CONCLUSIONS

In conclusion, it was exploring the muscular activation of the shoulder, elbow, and wrist muscles as they changed during the different phases of the various squash backhand stroke patterns. Particularly, the backhand straight had more AD muscle activation throughout the execution and follow-through phases than the backhand cross (p < .001). In contrast, muscular activity in the PD muscle was greater during the three phases of backhand cross than backhand straight (p < .001). There were no differences in the BB and TB muscles between the backhand cross and backhand straight in the muscles of the elbow. Furthermore, being the agonist muscle for the two types of backhand stroke, the TB muscle demonstrated the highest levels of activity during the execution phase. In this context, the wrist muscles perform in concert during the two patterns of backhand stroke phases by increasing the muscle activity of the WF muscle during the execution phase and transferring the load of activity to the WE muscle during the follow-through phase for control and more stability in the wrist joint. This knowledge of using electromyographic analysis on the main arm muscles may be useful in understanding muscular activation, optimizing performance, and preventing injury risks to the shoulder, elbow, and wrist during the phases of the two patterns of backhand stroke in squash.

AUTHOR CONTRIBUTIONS

Conceptualization, K.A., and A.-R.A.; methodology, K.A., and A.-R.A.; software, A.-R.A.; validation, K.A., A.A., and A.-R.A.; formal analysis, A.-R.A.; investigation, K.A., A.A., and A.-R.A.; resources, A.-R.A.; data curation, A.-R.A.; writing—original draft preparation, K.A., A.A., and A.-R.A.; writing—review and editing, K.A., A.A., and A.-R.A.; visualization, A.A., and A.-R.A.; supervision, A.-R.A.; project administration, A.-R.A. All authors have read and agreed to the published version of the manuscript.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki.

INFORMED CONSENT STATEMENT

Informed consent was freely obtained, and the study was approved by the institutional ethics committee of studies and research.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the Corresponding author.

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