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Research Article

The effect of eight weeks of aerobic training with curd consumption on the cortisol. Leptin and calcium levels in rural overweight Women

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Abstract

Background: Leptin, produced from obesity gene, plays a key role in weight regulation. Rural women are vulnerable due to some social restrictions. The purpose of this study was to investigate the effect of eight weeks of aerobic training along with curd consumption on cortisol, leptin and calcium levels in rural overweight women.

Materials and Methods: In this semi-experimental study, 32 overweight women with an age range of 30-40 years were randomly divided into four groups (control, supplement, training, training + supplement). The training program was running on a treadmill for 8 weeks, 3 sessions a week, each session 30 minutes with an intensity of 65% MHR in the first week and reached 45 minutes with an intensity of 70% MHR in the eighth week. 100 grams of curd for eight weeks, three days a week, were given to the supplemented groups. Blood samples were taken before and after the training program in the fasting state. Data were evaluated using multivariate covariance analysis ($P \leq 0.05$).

Results: The results showed that eight weeks of aerobic training with curd consumption caused a significant decrease in leptin, fat percentage and a significant increase in calcium in women (P=0.000). But it had no effect on cortisol.

Conclusion: aerobic training with curd can prevent injuries related to excess weight by improving fat metabolism and reducing leptin. To lose weight and maintain bone density in rural women, aerobic training along with curd consumption is recommended.

Keywords:
Aerobic training, curd, cortisol, leptin, calcium

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1. Introduction

Today, the importance of rural development and its place in the growth and development of countries is not hidden from anyone. Despite the importance of the role of women in the process of rural development, in different countries, especially developing countries such as Iran, rural women face many problems, including a lot of work in the fields and at home, little access to recreational facilities, limited freedom of action, Feeling insecure, weak communication skills, illiteracy or low literacy, long working hours and doing most of the agricultural activities, deprivation of receiving educational services and low level of health and other cases are prone to reduce the quality of life related to health [1]. Obesity and weight gain are closely related to the spread of diseases such as high blood pressure, increased blood lipids, and insulin resistance, which are all risk factors for diseases [2].

Leptin is a hormone that is secreted from adipose tissue and plays an important role in regulating energy homeostasis and increases with increasing obesity. After being released, this hormone is transported in the blood in a free form or attached to carrier proteins and by binding to receptors in the hypothalamus, it causes the release of neuropeptide Y and decreases appetite, also by increasing the activity of the sympathetic nervous system and increasing lipolysis, the body's metabolism rate increases and the amount of body fat is controlled [3]. Decreasing leptin concentration through exercise is associated with changes in energy balance, improvement in insulin sensitivity, changes in hormones related to carbohydrate and fat metabolism [4]. Sajadi and Shabani (2018) reported that 8 weeks of simultaneous aerobic and resistance training had a significant effect on the reduction of leptin and the level of anxiety in overweight and obese teenage girls, but no change was observed in the levels of cortisol [5].

Cortisol is one of the hormones associated with obesity and inactivity. Cortisol is a catabolism hormone that is secreted from the adrenal glands and some other tissues such as adipose tissue, liver and muscle and plays a role in the breakdown of proteins, triglycerides and free fatty acids (5). Cortisol is effective in regulating leptin levels by stimulating leptin gene expression and its secretion from fat cells. On the other hand, leptin reduces corticotropin-releasing hormone (CRH) and reduces the amount of stress and thus reduces the amount of cortisol secretion [6].

Inactivity causes a decrease in muscle mass and, subsequently, a decrease in flexibility and muscle strength, and causes a decrease in solutes and a lack of basic bone materials, so the process of bone density decreases and osteoporosis occurs [7]. On the other hand, extra weight causes cracks and fractures of the femor and hip bones, especially in obese people. Calcium plays an essential role in bone formation and strength [7]. Researchers know the presence of fat tissue in relation to the increase of inflammatory cytokines and tumor necrosis factor, which leads to the absorption of bone tissue [6, 5].

Experts in sports physiology and nutrition have always mentioned two factors of exercise and diet plan to reduce body fat, which have the greatest impact in combination [8]. Curd is a nutritious food that contains high quality protein, carbohydrates and electrolytes in addition to high calcium [8]. It also has little fat and significant amounts of phosphorus and iron [8]. Unlike other dairy products, this food not only does not cause digestive problems, but is also useful for indigestion and stomach acid balance.

Curd calcium limits the production of cortisol, and this prevents weight gain, and due to the presence of abundant leptin in protein materials, it causes a feeling of satiety and reduces the feeling of hunger, which can be a good supplement for obese or overweight people [8].

Due to the fact that today the majority of people are sedentary and obesity, overweight and stress are increasing and many diseases are spread due to inactivity and also the recommendation of the World Health Organization and doctors to do exercise to fight inactivity and disease and because dairy products are available and free of side effects compared to chemical drugs, and also because of the variety of training programs and the type of food and herbal supplements used in the research, which has led to contradictory and ambiguous results, the researcher considers to investigate the effects of eight weeks of aerobic training with curd consumption on cortisol, leptin and calcium levels in overweight rural women.

2. Materials and Methods

The current research is a semi- experimental type of research with code of ethics IR.IAU.ET.REC. 1401.018, Tehran branch of Islamic Azad University, which is compared in four groups (aerobic training, curd supplemented, aerobic training + curd supplemented and control), and with a pre- test and post- test design it placed. The statistical population of this research was all overweight women of Malekshahi city, 32 of them were voluntarily selected from Shohada ghaleh Jough sports club using Gpower software. The conditions for entering the research include having an age range between 30 and 40 years, no history of cardiovascular and musculoskeletal diseases, joint pain, diabetes and other acute and chronic diseases, no menstrual disorders, no menopause, no smoking, alcohol, medicine, and exclusion conditions. And the conditions for leaving the study included subjects' unwillingness to participate in exercises and taking supplements, missing more than one session in the exercise program, having any injuries caused by exercise and causing side effects with curd consumption.

Before starting the training protocol, in a briefing session, all plans, possible risks and the correct way of performing endurance exercises were explained to the participants. Also, the subjects were assured that their personal information will be kept confidential with the researcher. They were given the right to withdraw at any stage of the exercise if they do not want to continue cooperation. Written consent was obtained from all participants one week before at the beginning of the research program. The participants were examined by a trusted doctor to confirm their general health, cardiorespiratory health, control of not taking drugs, not having a specific disease, and not having movement problems.

Before starting the research protocol, anthropometric indicators of age, height, weight, body mass index, body fat percentage and maximum oxygen consumption of the subjects were measured. Body mass index was calculated in kilograms per square meter based on the height and weight of each person.

To measure the percentage of fat, a caliper (Yagami, made in Japan, with an accuracy of 0/2 mm) was used using a three-point method on the right side of the body. After measuring the thickness of subcutaneous fat in milliliters three times in a row, the average of the measurements obtained in three occasions was considered as the reference number. The sum of three point average measurements was put in Jackson and Pollock's equation in order to calculate body density.The body fat percentage of each subject was calculated using the obtained density [9].

Percentage body fat= ([4.95/db) -4/5] ×100

Body density (db) =1/099421-(0/0009929× S) + (0/0000023× S2) (0/0001392× age)

S=the total thickness of the subcutaneous fat of the triceps and Iliacus and thigh

Maximum oxygen consumption: The maximum oxygen consumption of the subjects was evaluated using a treadmill made in America and the standard Bruce test. According to the instructions, the Bruce test first started with a speed of 1/7 miles per hour and a 10% incline. After that, the incline and speed of the treadmill increased every three minutes. The test continued until the subjects reached exhaustion. At the end, based on the duration of running until reaching the stagnation stage and based on the following formula, the maximum oxygen consumption was determined in terms of mm/kg/ minute [9].

Maximum oxygen consumption= 14.76 - (1/379×time) - (0/451×The second power of time) -(0/012×The third power of time)

Training protocol: The training protocol was performed for eight weeks and three sessions per week on the treadmill. Aerobic exercises started from 30 minutes a day with an intensity of 65% of the target heart rate in the first week and reached 45 minutes with an intensity of 70% of the target heart rate in the eighth week. Before starting the exercise program, warm up for 10 minutes, including walking: 2 to 3 minutes, stretching exercises: 5 minutes; slow running: 2 to 3 minutes) and at the end a similar activity was considered for cooling [10].

It was performed under the supervision of a sports physiology expert in the gym. Heart rate during exercise through Karonen's formula was determined and adjusted based on a ratio of reserve heart rate [9].

Target heart rate= [Resting heart rate+ (maximum heart rate× percentage of desired intensity)] - resting heart rate

The maximum heart rate was determined using the following formula [9].

20***8*** ***-(0/7***×age)= maximum heart rate

The subjects' heart rate while running on the treadmill was monitored using a Beurer pm62 heart rate monitor.

Table 1.training protocol

training	week	intensity (Target heart rate percentage)	period of time (min)	Frequency
Aerobic training	1	65	30min/day	**3days/week**
	2	65	30min/day	**3days/week**
	3	65	35min/day	**3days/week**
	4	65	35min/day	**3days/week**
	5	70	40min/day	**3days/week**
	6	70	40min/day	**3days/week**
	7	70	45min/day	**3days/week**
	8	70	45min/day	**3days/week**

Supplement consumption: Subjects in the supplement groups consumed 100 grams of curd three days a week for eight weeks. Each 100 grams of curd contains 430 milligrams of calcium, 70.20 grams of protein, 7.10 grams of fat, 6 grams of water, 2 milligrams of iron and 378 kilocalorie energy [11]

48 hours before and after the training protocol and supplement consumption, the subjects' blood samples was collected in the amount of 5 cc per state 10- hour fasting from the brachial vein between 8 and 8:30 in the morning in the laboratory of Hakim Jurjani, of Malekshahi . After transferring to tubes containing EDTA and separating the plasma by a centrifuge (Routine 28 model with 4000 revolutions per minute, manufactured by Behdad Iran) was stored at 80 degrees until the time of measurement.

Leptin levels were measured using the Mercodia ELISA kit (Sweden country, with an accuracy of 0/5 ng/ ml) and cortisol using the IBL kit, made in Germany (internal variation coefficient 2-3% by ELISA method).

Calcium level with Pars test kit made in Iran and photometric laboratory method using ARSENAZO III was measured. Normal distribution was examined using the Kolmogorov- Smirnov test and to determine the homogeneity of variance Levine's test was used. Also, to check the significant changes of each of the research variables, between different groups, by the method of multivariate covariance analysis (MANCOVA) and if a statistically significant difference is observed, by Ben Feroni's post hoc test it was used to determine the location of differences between groups. A significance level of $P \leq 0/05$ was considered for all calculations. Statistical operations were performed using SPSS version 20 software.

3. Results

Characteristics of age, height, weight, body mass index, fat percentage and maximum oxygen consumption of subjects in pre- and post- test is presented in Table 2.

Table 2: Characteristics of subjects' age, height, weight, body mass index, fat percentage and maximum oxygen consumption

Vo2max (m1/kg/ min)	FAT (%)	BMI (kg/m^2)	Weight (KG)	HEIGHT (CM)	AGE (year)	Variable	group*
39.3±2.1	30.6 ± 0.9	27.1±4.3	71.2±4.7	161±2.02	37±2	Pretest	control
38.2±7.9	30.9 ± 0.47	27.1±5.2	71.2±7.5			posttest	
39.1±4.3	30.7 ± 0.48	27.2±6.2	72.4±7.5	162±2.3	38±3	Pretest	supplement
39.1±8.6	30.6 ± 0.69	27.2±2.6	71.5±5.7			posttest	
39.1±7.6	31.5 ± 0.53	28.2±7.05	71.5±8.3	160±2.06	39±2	Pretest	training
40.1±05.8	30.8 ± 0.65	26.2±2.2	67.5±5.2			posttest	
40.2±1.5	31.6 ± 0.87	28.1±8.02	77.1±4.9	163±2.9	39±2	Pretest	Training+ supplement
40.2±9.5	29.7 ± 0.85	26.95±4 .02	72.1±3.5			Posttest	

As can be seen in Table 2, the average values, weight, body mass index and fat percentage of all three experimental groups (supplement, training , training + supplement) compared to the control group decreased in the post- test, and the variable of fat percentage decreased in the supplement+ training group was significant compared to the other three groups (p=.003).

The amounts of cortisol, leptin and calcium hormones of the subjects in the pre- test and post-test are presented in Table 3.

Table 3. Cortisol, leptin and calcium levels of subjects before and after the test

Calcium(mg/dl)	Leptin(ng/ml)	Cortisol(ng/ml)	Variable	group*
±7/8./91	38/1±9/07	±3/9./87	**Pretest**	control
±04/8./607	39/1±2/1	±08/9./95	**posttest**	
±5/8./87	38/1±8	9/1±5/03	**Pretest**	supplement
±4/10./72	±3/37./99	±3/9./72	**posttest**	
9/1±2/1	39/1±1/2	±8/9./84	**Pretest**	training
±03/10./93	37/1±1/7	9/1±8/1	**posttest**	
9/1±2/01	±2/39./68	±3/9./91	**Pretest**	Training supplement
11/10±3/1	36/1±01/07	±7/9./508	**posttest**	

The average values of all three experimental groups in the leptin variable had a significant decrease compared to the control group in the post- test (P=0/000, effect size= 0/799) and between the supplement group and the training + supplement group (p= 0.001).

The average amount of calcium in all three experimental groups compared to the control group increased significantly in the post- test (p=0/000, effect size =0/783), and between the training group and the training + supplement group (p = 0.001) and supplement group (p=0.001) there was a statistically significant difference was observed. No significant difference was observed in the cortisol variable (P=0.536).

4. Discussion

The results of the present study showed that there was no significant change in the cortisol level of overweight rural women as a result of eight weeks of aerobic training and curd consumption. Sari Saraf et al (2019) in a research showed that eight weeks of aerobic training does not have a significant effect on the cortisol levels of obese women [12]. In a study, Khorshidi et al (2013) showed that 12 weeks of increasing aerobic training did not significantly change the amount of cortisol in obese men [13], which is consistent with the results of the present research. On the other hand, Suri et al (2016) found that cortisol decreased significantly in obese men after 12 weeks of endurance training (14). It seems that the reduction of cortisol can play an effective role in reducing the catabolic state of the body and cause physiological adaptations to increase physical performance and improve the body composition of overweight people. Cortisol changes due to exercise depend on different factors.

Cortisol concentration changes with exercises that are performed with an intensity of more than 65% of the maximum oxygen consumption (15). Changes in body plasma volume as a result of exercises most of them lead to the loss of water and the change of electrolytes in the athlete's body, as well as the relative humidity and the change in the temperature of the environment also contribute to the change in cortisol concentration (16). Tanskanen et al (2011), the decrease in cortisol concentration during four weeks and the increase in cortisol concentration reported during eight weeks of training with sub- maximal intensity (17), in general, the creation of cortisol response to training depends on factors such as the involvement of large muscle mass, the intensity and volume of training (14). The use of curd supplement did not cause a significant change in the serum cortisol of rural overweight women. In line with the results of the present study, in the research of Pourabdi et al. (2018), consumption of 500 ml of low-fat milk did not significantly change the response of cortisol and CRP after resistance training in healthy young women (18).

The present study showed that as a result of eight weeks of aerobic training and consumption of curd, the level of leptin in rural overweight women decreased significantly. With the results of the present study, Rostamizadeh et al (2017) reported a significant decrease in serum leptin as a result of eight weeks of aerobic and resistance training in overweight men(19). Sheikh Qalavand (1400) reported a significant decrease in serum leptin in overweight women as a result of eight weeks of Pilates training (20). on the other hand, Khajelandi et al (1396) reported that leptin in inactive overweight women did not change significantly after eight weeks of Pilates training (21) .

Khanvari et al (1400) also reported no significant change in leptin in adolescent boys with reported overweight as a result of eight weeks of intense intermittent training (22). It is believed that the leptin of overweight and obese adolescents is affected by other effective factors such as hormonal factors due to being in the puberty period and sports training during this period cannot overcome the effects of other factors. Among the contradictions in these results, we can mention the difference in the training programs, subjects and especially the intensity and duration of the exercises. Fat burning requires more oxygen. As a result of aerobic exercises, the capillary density increases, which means that more blood and oxygen reach the muscle tissue, and during long- term submaximal activities, when more oxygen is available, it uses fat metabolism to meet the required energy (12). Leptin is derived from fats, therefore, with the reduction of body fat, the amount of leptin also decreases. As in the present study, the weight and body mass index of overweight women decreased. Some studies have shown that physical activity does not only decrease leptin levels it reduces fat mass by increasing leptin sensitivity.

One of the other mechanisms is the increase in the function of glucose transporter 4 in sports activities, which causes glucose to enter fat cells through GLUT4. Glucose acts as an intracellular signal and stimulates the release of leptin from fat cells (14). Performing physical exercises increases the amount of cotcholamines, which which has an inverse relationship with the secretion of leptin and causes a decrease in the level of leptin (23). Hormones such as sex hormones and thyroid hormones play a role in leptin regulation. These hormones are responsible for the production of obesity by regulating the woman Cortisol and growth hormones are the most important hormones that increase the amount of leptin secretion (23).

As mentioned, stress caused by exercise, changes in fuel transfer, concentration of systemic hormones, amount of energy consumed, and reduction of fat mass are among the reasons that change leptin levels (4). According to the studies, short- term exercise cannot affect leptin secretion, but short- term changes in energy balance caused by exercise have an effect on nighttime leptin secretion. Negative energy balance suppresses nocturnal leptin secretion. If the positive energy balance leads to an increase in leptin secretion (4).curd consumption also caused a significant decrease in serum leptin in overweight rural women, but the synergistic effect of aerobic exercise and curd consumption caused a further decrease in women's leptin. Mousovian et al (2019) stated that the consumption of dairy products caused a significant decrease in serum leptin in adults (24) Dow and colleagues (2010) also found that consuming 600 ml of milk compared to 600 ml of fruit juice at breakfast increased the sensation Satiety and fullness, decrease in appetite and decreased energy intake at lunch in overweight women and men (25).

Consuming wrong foods contain high amounts of fat or sugar, as well as high calcium and protein content and other dairy foods affect appetite and energy consumption. Evidence from studies show that consumption dairy products increase fat metabolism, several anti-appetite hormonal peptides are released from the digestive system, and appetite affects and improves mental satiety and energy consumption (26) It seems that the significant decrease in appetite and energy after drinking milk can be attributed to the presence of proteins in milk. Studies have shown that whey protein in milk has a short- term satiety effect, but casein protein affects the feeling of satiety in the long term due to slower gastric emptying (26).

Calcium in milk can also be another factor of loss of appetite. However, Haji Mohammadi et al (2017) stated in a research that the effect of consumption of foods rich in vitamin D (yogurt) significantly increased the level of leptin in people with type 2 diabetes (27).

The results of this study showed that after eight weeks of training and curd consumption found a significant increase in the calcium level of rural overweight women. Mofidi Sadr et al (2018) in a research showed a significant increase in serum calcium as a result of a combined resistance- aerobic training course in obese and overweight postmenopausal women (28). Alghadir et al (2014), who investigated the effect of 12 weeks of aerobic training on 36 men and 29 women, observed a significant increase in bone metabolic indices such as alkaline phosphatase, osteocalin, calcium and bone density in the subjects (29) .in contrast to Zargar et al (2016) stated that three months of aerobic training did not have a significant effect on the calcium concentration of obese men (30).

Participating in aerobic activities causes a lot of energy consumption. In endurance activities of repeated muscle contractions in order to continue the activity and release the needed energy, it is strongly dependent on the presence of calcium during endurance activities. A large amount of body salts that calcium being one of them, is excreted through the skin and during the sweating process therefore, blood calcium decreases (30). Because the level of blood calcium must always remain constant, so hormonal systems sensitive to blood calcium levels are activated (28). Researchers believe that changes in plasma calcium are mostly related to hormonal factors that regulate calcium levels. Parathormone is one of the important factors in regulating bone metabolism.

The most important function of this hormone is to maintain the homeostasis of inorganic calcium phosphate ion through protein related receptor in kidney, bone and intestine. Parathormone increases plasma calcium levels by stimulating calcium reabsorption in the intestine and increasing bone reabsorption. By increasing the production of 25-hydroxyvitamin D3 in the kidney, this hormone increases the absorption of calcium in the kidney, and sports activities cause better absorption of calcium from the digestive system, and on the other hand, the presence of sufficient amounts of calcium causes a decrease in the production of parathormone hormone and hence bone removal calcium, this affects the positive changes of the bone fence. On the other hand, increasing the presence of phosphate ions also causes an increase in parathormone levels (30).Aerobic training can reduce the circadian rhythm of this hormone. Interval training with sufficient intensity has more effect on the reduction of parathormone, which itself is the reason for the optimal effect on bone density (28).

Curd consumption caused a significant increase in calcium in overweight rural women. Curd is an excellent protein in terms of nutrition and the denser it is, the amount of protein per unit weight is increased so that its protein can be compared with meat. In addition to protein, curd is rich in calcium, phosphorus and a group of B vitamins and micronutrients such as iron and zinc. The lower the moisture content of the curd and the less processing done on it, the higher the nutrients, especially calcium (8).

The results of the present study showed the fat percentage of overweight women in rural areas was significantly reduced. At rest, 50% of the energy required for body metabolism is provided by fats.

In moderate sports, fats are responsible for providing fuel, but with the increase in the intensity of sports activity, the metabolism of sugars increases, so moderate activity is more suitable for weight loss. A moderate intensity training program such as slow running can increase changes in body fat It causes a small decrease in fat- free weight and a small decrease in total body weight (31) due to aerobic activity, the ability to harvest and oxidize fat in trained muscles increases. In these exercises, with the increase in the activity of the lipoprotein lipase enzyme, the beta oxidation capacity of fat in the muscle increases, and its important effect is to increase the proportion of fat and, as a result, reduce the proportion of glucose in creating energy in aerobic exercise. As a result of aerobic activities, due to the increase in mitochondrial density, the capacity of oxidative enzymes of trained muscle fibers increases compared to the resting muscle. In addition to the increase in the activity of the enzymes of the electron transport chain, the activity of the enzymes involved in the oxidation of fats also increases (21) .Sabzevari Rad et al (2020) in a research, they stated that eight weeks of aerobic training caused a significant reduction in weight, body mass index and body fat percentage in obese and overweight officer students (32).

Ange et al (2021) reported in a research that 12 weeks of physical activity in people with metabolic syndrome significantly reduced waist circumference, blood glucose, triglycerides, body mass index, subcutaneous fat, low density lipoprotein (33) vs Torabi et al (2015) reported eight weeks of endurance training along with the consumption of cinnamon supplement, it did not have a significant effect on the body fat percentage of women with type 2 diabetes (34).

It is possible that the contradictory finding can be explained in the difference in intensity, volume and duration of the exercise program of combined exercises versus aerobic exercises, control or lack of control of the index diet. Body mass, age, physical condition, and disease conditions of the subject.

High calcium in the diet reduces the level of calcitriol, so it causes a decrease in intracellular calcium and ultimately leads to the stimulation of lipolysis. Low intracellular calcium prevents the expression of fatty acid synthase enzyme complex; therefore, calcium intake can have a direct effect on the storage and breakdown of fat in adipose tissue (25). Consumption of curd also caused a significant decrease in body fat percentage in the present study. Same with the results of the present study, Vitbrak et al (2013) reported in a study that consumption of dairy products reduced weight and fat percentage in overweight women (35).

Conclusion

Based on the results of the current research, probably eight weeks of moderate intensity aerobic training and curd consumption as one the non- invasive method leads to the improvement of fat metabolism conditions in order to prevent diseases caused by excess weight. Obesity, such as heart disease and diabetes in obese women, can be improved by reducing leptin concentration, with changes in energy balance. Insulin sensitivity is associated with changes in hormones associated with carbohydrate and fat metabolism. There is a negative relationship between dairy consumption and metabolic disorders. Dairy products cause short- term suppression of food consumption, increase mental satiety, and stimulate mechanisms known to send satiety and satiety signals (36).

A regular body activity along with the consumption of curd leads to an increase in bone density and a decrease in the risk of fractures in overweight women. Considering the small number of subjects in this research and the lack of control of the subjects' nutritional and psychological factors, as well as the short period of the research protocol to reach for accurate results, it is better to carry out more researches in accordance with the mentioned cases in the field of the effect of training and consumption of dairy products in overweight people.

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Compliance with ethical standards

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Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: Z.E, M.H, A.R.I; Methodology: Z.E, M.H, A.R.I; Software: M.H., M.A.A., M.P., S.A.H.; Z.E, M.H, A.R.I; Validation: Z.E, M.H, A.R.I; Formal analysis: Z.E, M.H, A.R.I; Investigation: Z.E, M.H, A.R.I; Resources: Z.E, M.H, A.R.I; Data curation: Z.E, M.H, A.R.I; Writing - original draft: Z.E, M.H, A.R.I; Writing - review & editing: Z.E, M.H, A.R.I; Visualization: Z.E, M.H, A.R.I; Supervision: Z.E, M.H, A.R.I; Project administration: Z.E, M.H, A.R.I; Funding acquisition: : Z.E, M.H, A.R.I;

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