

Original Article

Keap1 Gene Variants (rs11085735) and Lipid Profile in Obese Individuals From Kurdistan, Iraq

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Abstract

Background: Obesity is defined as excessive fat accumulation in the adipose tissue and peripheral organs. The Kelch-like ECH-associated protein 1 (Keap1) is involved in cellular protection in obesity.

Objectives: The objectives of this study were to detect the association of Keap1 rs11085735 variants with the risk of obesity in relation to anthropometric parameters and lipid profile in a population from the Kurdistan of Iraq.

Methods: A total of 265 volunteers (134 females and 131 males) were randomly selected. According to the body mass index (BMI), individuals were divided into subjects with normal BMI (131 healthy individuals) and obese subjects (134 obese volunteers). Anthropometry and lipid profile parameters were measured in these volunteers and Keap1 rs11085735 variants were identified by the polymerase chain reaction (PCR) followed by digestion with HinfI restriction enzyme.

Results: Triglycerides and low-density lipoprotein cholesterol (LDL-C) serum levels were significantly higher ($P < 0.001$) in the obese group compared to the control group. The serum level of high-density lipoprotein cholesterol (HDL-C) was significantly lower ($P = 0.02$) in the obese individuals than in the controls. Moreover, the waist, hip, and wrist circumferences and the waist to hip ratio in obese individuals were significantly higher than in controls ($P < 0.001$). The frequency of Keap1 AA genotype was 1.5% in the obese group and 0% in the controls ($P = 0.36$). The frequency of A allele was 4.9% and 3.1% in obese subjects and controls, respectively ($P = 0.28$). Considering all individuals, carriers of AA genotype of Keap1 had significantly higher BMI and waist and hip circumferences compared to the carriers of AC and CC genotypes.

Conclusion: The present study found abnormal lipid profile among obese individuals compared to individuals with normal BMI. Our findings indicated that the presence of Keap1 polymorphism influenced the anthropometric parameters, and the carriers of AA genotype of Keap1 had significantly higher BMI and waist and hip circumferences compared to the carriers of AC and CC genotypes.

Keywords: Obesity, Keap1 variants, Anthropometry

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Background

The prevalence of obesity as a global problem is increasing in the world (1,2). Worldwide, there are 1 billion overweight individuals and 300 million obese persons. It is estimated that in 2030, 2 billion individuals will be overweight and there be 1.12 billion obese subjects around the world (3). Obesity, an excess amount of body fat, is a major risk factor for mortality (4). The risk of mortality from cardiovascular diseases related to obesity was 13%, and a body mass index (BMI) equal or more than 25 kg/m² could enhance the risk of all-cause mortality to around 30% (5). The prevalence of obesity as a chronic inflammatory disease is enhancing. Ectopic and the excess accumulation of fat may result in adverse health effects and decreased life span. BMI, defined as the weight (kg)/height

squared (m²), is commonly used to identify underweight individuals with a BMI equal or lower than 18.5 kg/m², normal weight subjects with a BMI of 18.5-24.9 kg/m², overweight individuals with a BMI of 25-30 kg/m², and obese adults with a BMI equal or more than 30 kg/m² (6).

Ectopic and excess fat accumulation leads to adverse health effects and decreased life span. Individuals with BMI exceeding 30 kg/m² are considered obese. However, BMI does not evaluate the lean-to-fat mass ratio. A more precise evaluation of adiposity is performed by calculating the body fat distribution by measurement of the waist circumference or waist-to-hip ratio (7). The high mortality and morbidity rates related to obesity are largely attributed to cardiovascular complications (8). Obesity and obesity-associated metabolic and cardiovascular risk



factors are designated as cardio-metabolic syndrome. These risk factors include hypertension, dyslipidemia, and type 2 diabetes mellitus (T2DM) (9).

Obesity can be affected by many factors including genetic background and lifestyle (10). The signaling pathway of nuclear factor erythroid 2-like 2 (Nrf2)/Kelch like ECH associated protein 1 (Keap1) protects cells, tissues, organs, and systems from oxidative stress. Therefore, Nrf2 is known as a multi-organ protector (11,12). This pathway is involved in antioxidant defense mechanisms in cardiovascular diseases including atherosclerosis and hypertension (13,14). In the pathway, where Nrf2 is the main regulator of oxidant/antioxidant balance, the cellular response is controlled to prevent injury to redox-sensitive cellular components (14). The Nrf2 transcription factor is tightly regulated by the cytoplasmic repressor Keap1. Keap1 plays a key role in the degradation of Nrf2 by the pathway of ubiquitin–proteasome (15). In oxidative stress, Nrf2 separates from Keap1, translocates to the nucleus, and activates many cytoprotective genes against the oxidative stress (16,17).

The polymorphism of Keap1 (A/C) rs11085735 is an intron variant which does not directly affect an amino acid sequence and protein structure. However, this polymorphism is located in the intron 2 of the Keap1 gene relatively close to the exon 3 of the gene (73-bp); therefore, it might have functional consequences by affecting the splicing of the Keap1 mRNA (18) and influence the structure of Kelch-1 domain and the interaction of Keap1 with Nrf2 (19). The frequency of the minor A allele of Keap1 is different in various populations. The frequency of A allele was found to be 7% in Europeans and 12.4% in Africans. Its overall frequency among Asians was reported to be 0.1%, ranging from 0% in East Asian to 2.6% in South Asian populations (20). Previously, we reported a frequency of 4% for A allele of Keap1 among healthy individuals with Kurdish ethnic background from Western Iran (21)

This study aimed to detect the frequency of Keap1 rs11085735 gene variants in obese individuals compared to healthy subjects with normal BMI and to find a possible association between this polymorphism and the risk of obesity in relation to anthropometric characteristics and lipid profile among a population from Sulaimani, Iraq.

Materials and Methods

Study Design

This case-control study enrolled a total of 265 individuals from Sulaimani, Kurdistan, Iraq. The sample consisted of 134 females and 131 males. The 265 volunteers were classified into two groups according to their BMI, including obese individuals (BMI \geq 30 kg/m²) and age- and gender-matched healthy individuals with normal BMI (BMI of 18.5 to 24.9 kg/m²). They were referred to the public health laboratory in the teaching hospital Sulaimani from May to September 2021. The volunteers were apparently healthy people who were accompanying patients, apparently

healthy obese volunteers, and apparently healthy staff of the laboratory. Pregnant women and patients who had blood transfusion or surgery in the last three months were excluded from the study. The studied individuals were not smokers or alcohol consumers. Additionally, they had a mixed diet including meat and vegetables and most of them did not have physical activity. Healthy individuals with 20 to 59 years of age and obesity were selected for the present study. The exclusion criteria were smoking, alcoholism, neuropsychiatric disorders, consumption of anorexigenic agents or anabolic steroids, hypothyroidism, polycystic ovarian syndrome, chronic diseases, and pregnancy or plans to become pregnant during the study period. Participants with a history of hypertension, diabetes, kidney disease, and/or cardiovascular diseases were excluded from the study. Anthropometric parameters including height, weight, and waist, hip, and wrist circumferences were measured. BMI was measured by dividing the weight (kg) by the height squared (m²) and accordingly, a BMI of \geq 30 kg/m², based on the World Health Organization classification, was used to define obesity.

Sampling

First, 6 mL of fasting venous blood sample was obtained in the morning from individuals with normal BMI and obese individuals. Then, the blood samples were collected in EDTA tubes (3 mL) for DNA extraction and serum gel separator tubes (3 mL), which were allowed to clot and settle at room temperature while participants were in sitting position during blood aspiration. The serum was separated immediately from blood samples by centrifugation at 4000 rpm for 10 minutes.

Analysis of Biochemical Parameters

The serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were measured by standard enzymatic methods using an automated Biochemistry Analyzer [KENZA 450 TX (BIOLABO Diagnostics), France].

Genotyping

DNA extraction from the peripheral blood leukocytes was done using the standard phenol–chloroform method. The DNA content of the samples was measured and the purity of DNA was assessed by a NanoDrop spectrophotometer. Using the polymerase chain reaction (PCR), DNA was amplified. The PCR products were digested with HinfI restriction enzyme, using the restriction fragment length polymorphism (RFLP) method, to identify the genotypes of the Keap1 A/C (rs11085735) gene. Briefly, DNA was amplified using the PCR method by the specific primers of P1: 5' GGT CAC TGA CTA GAA CTC TCC AAG G 3' (the forward primer) and P2: 5' GCT GCA TCC ACC ACA ACA GTG T 3' (the reverse primer). PCR reaction (20 μ L) included 1 μ L of DNA (200–600 ng), 1 μ L of each primer (100 pmol), 7 μ L of ddH₂O, and 10 μ L of master

mix 2X that contained MgCl₂, dNTPs, PCR buffer, and Taq DNA polymerase. The optimized ready-to-use Master Mix was used for the amplification of DNA. The PCR process was done in 35 cycles. It started with initial denaturation (95°C for 5 minutes), denaturation (95°C for 30 seconds), annealing step (62.5°C for 35 seconds), and extension step (72°C for 40 seconds). Extension for 10 minutes at 72°C was used as the final extension step. The obtained PCR product (192-bp) was digested with HinfI. The PCR product was not cleaved by HinfI restriction enzyme in samples with AA genotype and a fragment with 192-bp was detected. Three fragments (192-bp, 104-bp, and 88-bp) were produced in the AC genotype after digestion of PCR products with HinfI, and 104-bp and 88-bp fragments were detected in the presence of the CC genotype (Figure 1).

Statistical Analysis

The SPSS version 16.0 was used for statistical analysis. The frequency of Keap1 genotypes was compared between two groups using the χ^2 test and Pearson correlation coefficient. The values of quantitative parameters were compared between the two groups using *t* test. The analysis of variance (ANOVA) was used for comparing the anthropometrical parameters between three genotypes of Keap1. $P < 0.05$ was considered statistically significant.

Results

Anthropometry parameters of the studied subjects are depicted in Table 1. A total of 265 individuals including 131 controls with normal BMI (23 ± 1.8 kg/m²) and 134 obese subjects with a mean BMI of 35.7 ± 4.9 kg/m² ($P < 0.001$) were studied. Controls had a mean age of 38.9 ± 8.7 years and the mean age of obese individuals was 40.7 ± 10.2 years ($P = 0.13$). As shown in Table 1, the mean waist, hip, and wrist circumferences and waist-to-hip ratio in the obese group were significantly higher compared to controls. The mean serum level of triglycerides was 151.3 ± 86.9 in the obese group and it was 111.6 ± 56.5 mg/dL in the control group ($P < 0.001$). The mean LDL-C level was 126.7 ± 34.2 in the obese group and it was 105.9 ± 30.6 mg/dL in controls ($P < 0.001$). The mean serum HDL-C level was 44.7 ± 11.3 in obese individuals and it was 48.0 ± 11.8 mg/dL in controls ($P = 0.02$) (Table 1).

Characteristics of obese individuals and controls

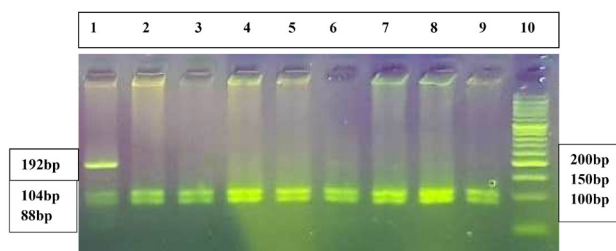


Figure 1. The 3% Agarose Gel Electrophoresis of Amplified Keap1 Gene Digested with HinfI Restriction Enzyme. Lane 1 indicates the AC genotype. Lanes 2-9 demonstrate the CC genotype. Lane 10 shows the 50-bp DNA molecular weight marker

Table 1. Characteristics of Obese Individuals and Controls With Normal BMI

Variables	Obese individuals (n = 134) Mean \pm SD	Controls (n = 131) Mean \pm SD	P value
Age (years)	40.7 \pm 10.2	38.9 \pm 8.7	0.13
Range	20-59	20-59	
BMI (kg/m ²)	35.7 \pm 4.9	23 \pm 1.8	<0.001
Waist circumference (cm)	109.3 \pm 11	83.9 \pm 7.5	<0.001
Hip circumference (cm)	119.8 \pm 9.5	97.2 \pm 4.8	<0.001
Wrist circumference (cm)	17.8 \pm 1.2	16 \pm 1.2	<0.001
Waist to hip ratio	0.91 \pm 0.07	0.86 \pm 0.05	<0.001
Total cholesterol (mg/dl)	182.6 \pm 39.3	174 \pm 38.5	0.07
Triglycerides (mg/dl)	151.3 \pm 86.9	111.6 \pm 56.5	<0.001
HDL-C (mg/dl)	44.7 \pm 11.3	48 \pm 11.8	0.02
LDL-C (mg/dl)	126.7 \pm 34.2	105.9 \pm 30.6	<0.001

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

Table 2. Characteristics of Obese Individuals and Controls According to Gender

Variables	Obese individuals (n = 134) Male = 66, Female = 68 Mean \pm SD	Controls (n = 131) Male = 65, Female = 66 Mean \pm SD
Age (years)		
Male	40.6 \pm 10.5, $P = 0.37$	38.7 \pm 9.3
Female	40.7 \pm 9.9, $P = 0.22$	39.2 \pm 8
BMI (kg/m ²)		
Male	34.4 \pm 3.6, $P < 0.001$	23.4 \pm 1.6
Female	37 \pm 5.6, $P < 0.001$	22.6 \pm 1.9
Waist circumference (cm)		
Male	112.6 \pm 9.9, $P < 0.001$	88.4 \pm 5.5
Female	106 \pm 11, $P < 0.001$	79.5 \pm 6.6
Hip circumference (cm)		
Male	117.1 \pm 6.7, $P < 0.001$	98.6 \pm 4.4
Female	122.5 \pm 11, $P < 0.001$	95.9 \pm 4.8
Wrist circumference (cm)		
Male	18.5 \pm 0.85, $P < 0.001$	16.9 \pm 0.87
Female	17.1 \pm 1.1, $P < 0.001$	15.2 \pm 0.8
Waist: hip ratio		
Male	0.96 \pm 0.05, $P < 0.001$	0.89 \pm 0.05
Female	0.87 \pm 0.05, $P < 0.001$	0.83 \pm 0.05
Total cholesterol (mg/dL)		
Male	188.3 \pm 39.8, $P = 0.003$	167.8 \pm 39.1
Female	177 \pm 38.4, $P = 0.61$	180 \pm 37.1
Triglycerides (mg/dL)		
Male	175.9 \pm 101.3, $P = 0.002$	128.7 \pm 61.7
Female	127.4 \pm 62, $P = 0.001$	94.8 \pm 45.4
HDL-C (mg/dL)		
Male	42.3 \pm 9, $P = 0.84$	42 \pm 8.2
Female	47.1 \pm 9, $P = 0.002$	54 \pm 11.8
LDL-C (mg/dL)		
Male	129.3 \pm 36.9, $P < 0.001$	103.9 \pm 31
Female	124.1 \pm 31.4, $P = 0.003$	107.8 \pm 30.3

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

according to the gender are demonstrated in Table 2. Waist, hip, and wrist circumferences and the mean waist-to-hip ratio in both males and females in the obese group were significantly higher compared to those in the controls (Table 2). The mean serum level of total cholesterol in obese males was significantly higher than that in males with normal BMI. The mean serum level of triglycerides in both obese males and females was significantly higher than in males and females with normal BMI. Additionally, the LDL-C level in both obese males and females was significantly higher compared to males and females with normal BMI. However, the serum level of HDL-C in obese women was significantly lower than that in women with normal BMI (Table 2).

Table 3 demonstrates the distribution of Keap1 rs11085735 genotypes and alleles in obese individuals and subjects with normal BMI. The Keap1 AA genotype frequency was 1.5% in the obese group and it was 0% in the control group ($P=0.36$). The A allele frequency was 4.9% in obese individuals and it was 3.1% in the controls ($P=0.28$).

Anthropometric and lipid parameters were studied among various genotypes of Keap1 in all studied individuals and in obese subjects that are indicated in Tables 4 and 5. As indicated in Tables 4 and 5, carriers of the AA genotype had a significantly higher BMI than the carriers of AC and CC genotypes considering all

Table 3. Distribution of Keap1 Genotypes and Alleles in Obese Individuals and Controls

Keap1 Parameters	Obese individuals No. (%)	Controls No. (%)	P Value
Genotypes			
AA	2 (1.5)	0 (0)	0.36*
AC	9 (6.7)	8 (6.1)	
CC	123 (91.8)	123 (93.9)	
Alleles			
A	13 (4.9)	8 (3.1)	0.28
C	255 (95.1)	254 (96.9)	

*Overall P value comparing 3 genotypes.

Table 4. Association of Studied Parameters with Keap1 Genotypes in All Individuals

Variables	AA	AC	CC	Overall P Value
BMI (kg/m ²)	40.9±2.7	31.3±9.3	29.2±7.1	0.044
Waist circumference (cm)	122±11.3	101.7±19.8	96.2±15.3	0.028
Hip circumference (cm)	131.5±6.4	111.6±17.2	108.3±13.2	0.035
Wrist circumference (cm)	18.8±1.1	17.2±1.2	16.9±1.5	0.13
Waist-to-hip ratio	0.93±0.04	0.91±0.07	0.88±0.07	0.34
Total cholesterol (mg/dL)	146±49.5	169.8±27	179.2±39.7	0.31
Triglycerides (mg/dL)	109±11.3	123.4±51	132.4±77.7	0.81
HDL-C (mg/dL)	40±17	45.1±10.8	46.5±11.7	0.66
LDL-C (mg/dL)	116±52.3	111.8±23.8	116.7±34.6	0.84

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

studied individuals. In addition, the carriers of the AA genotype had significantly higher waist circumference compared to carriers of the AC and CC genotypes among all individuals and obese people. Further, in the presence of AA genotype, a significantly higher hip circumference was detected in all studied subjects and obese individuals compared to AC and CC genotypes (Tables 4 and 5).

Discussion

We detected abnormal lipid profile among obese individuals compared to controls. In other words, significantly higher levels of triglycerides and LDL-C and lower HDL-C levels were found in the obese group than the controls. Moreover, in obese men, total cholesterol level was significantly higher compared to the normal BMI group. However, a significantly lower level of HDL-C was found in obese women compared to controls. Obesity, especially the accumulation of visceral fat, enhances hypertension, dyslipidemia, and the risk of T2DM, contributes to cardiovascular diseases, reduces life quality, and increases medical care costs and premature mortality (22,23). Obesity induces metabolic syndrome complications and glucose and lipid metabolism disorders. It has been reported that obesity is associated with increased levels of total cholesterol, LDL-C, and TG and decreased HDL-C levels. Moreover, the increased prevalence of obesity is accompanied by an increase in the prevalence of diabetes and pre-diabetes (24).

The presence of the minor A allele of Keap1, rs11085735, results in the lower expression of Keap1, higher expression of Nrf2, and reduced overall survival of patients with breast cancer treated with tamoxifen and radiotherapy (19). The Nrf2-Keap1 system protects pancreatic beta cells against oxidative stress through multiple pathways and it is necessary for the control of glucose metabolism by the secretion of insulin and utilization of glucose in insulin-sensitive tissues (25). There is no available study to investigate the frequency and the effect of Keap1 gene polymorphism rs11085735 on the risk of obesity. In our study, a higher AA genotype frequency was observed in obese individuals than

Table 5. Association of Studied Parameters With Keap1 Genotypes in Obese Individuals

Variables	AA	AC	CC	Overall P value
BMI (kg/m ²)	40.9±2.7	38.6±6.7	35.4±4.7	0.052
Waist circumference (cm)	122±11.3	116.2±15.7	108.5±10.3	0.031
Hip circumference (cm)	131.5±6.4	124.9±12.3	119.5±9.1	0.048
Wrist circumference (cm)	18.8±1.1	17.9±1	17.7±1.2	0.46
Waist-to-hip ratio	0.93±0.04	0.93±0.09	0.91±0.07	0.73
Total cholesterol (mg/dL)	146±49.5	167.1±29.3	184.3±39.7	0.19
Triglycerides (mg/dL)	109±11.3	120.2±39.2	154.3±89.5	0.41
HDL-C (mg/dL)	40±17	46.3±12.7	44.7±11.2	0.76
LDL-C (mg/dL)	116±52.3	111.6±26.9	127.9±34.4	0.34

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

in controls, which was not significant. However, the Keap1 polymorphism affected the anthropometric parameters as the carriers of AA genotype had significantly higher BMI and waist and hip circumferences compared to the carriers of AC and CC genotypes.

In obese individuals, there is a redox imbalance and potentiated oxidative stress. The Nrf2- Keap1 pathway is necessary for the prevention of the metabolic dysregulation linked to obesity (26). During oxidative stress, Keap1 acts as a sensor of redox homeostasis and ROS reacts with Keap1 and modifies the Keap1 homodimer structure, resulting in Nrf2 release. After translocation into the nucleus, the released Nrf2 induces antioxidant response-related gene expression (26). Therefore, genetic variation in Nrf2 and its related genes could be associated with the development of obesity. The importance of Nrf2 in insulin resistance and obesity suggested the potential therapeutic use of Nrf2 activators (27).

Several studies indicated the association of BMI and T2DM with the increased risk of T2DM as the BMI exceeds 25 kg/m². A systematic review of 18 weight-related diseases suggested that diabetes mellitus is one of the most widespread metabolic diseases and men with BMI ≥ 30 kg/m² had a 7 times and women with BMI ≥ 30 kg/m² had a 12 times higher risk of T2DM development than men and women with normal BMI (BMI ≤ 25 kg/m²) (6). Furthermore, fat distribution and the relative proportions of lipids in different insulin-sensitive tissues, skeletal muscle, adipose tissue, and liver play a major role in the pathophysiology of obesity.

Waist circumference, a measure of abdominal obesity, is considered a better predictor of T2DM. Insulin resistance along with the dysfunction of pancreatic β-cells is a risk factor for the development of T2DM (28).

One of the most important and dangerous forms of obesity is abdominal obesity. Intra-abdominal or visceral fat accumulation is the main characteristic of metabolic syndrome that is associated with a higher incidence of diabetes mellitus and cardiovascular disease (29).

The International Diabetes Federation has considered the gender effect on the waist circumference-based risk, by adding the ethnicity into the definition of central obesity as a metabolic syndrome component (30). Estimating the waist circumference has been the better anthropometric measurement of abdominal adiposity. Measurement of abdominal adiposity is useful for the assessment of insulin resistance and risks of coronary heart disease in various populations. Measurement of the waist circumference could be useful in identifying subjects at an increased risk of insulin resistance and coronary heart disease (31). Visceral fat accumulation increases pro-oxidant and pro-inflammatory states. Animal studies along with epidemiological, and clinical reports suggested that oxidative stress is involved in the pathogenesis of obesity and its associated risk factors (32).

Conclusion

Our study found an abnormal lipid profile in obese individuals compared to individuals with normal BMI. In the present study, a higher Keap1 AA genotype frequency was observed in obese individuals compared to controls, which was not significant. However, the Keap1 polymorphism influenced the anthropometric parameters and the carriers of Keap1 AA genotype had significantly higher BMI and waist and hip circumferences compared to the carriers of AC and CC genotypes.

Limitation of the Study

The use of a small sample size in studying the association of anthropometric and lipid profile parameters with the Keap1 AA genotype, due to the low frequency of this genotype, was the limitation of the study.

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Project administration: Zohreh Rahimi.

Resources: Avan Arif Ahmad, Zohreh Rahimi.

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Validation: Zohreh Rahimi, Avan Arif Ahmad.

Visualization: Zohreh Rahimi, Asad Vaisi-Raygani.

Writing – original draft: Zohreh Rahimi, Avan Arif Ahmad.

Writing – review & editing: Zohreh Rahimi, Avan Arif Ahmad, Asad Vaisi-Raygani.

Conflict of Interests

There are no conflicts of interest.

Ethical Issues

The research was performed according to the Helsinki Declaration. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1400.7141). Written informed consent was obtained from all participants before the study. Authors have completely observed ethical issues such as data fabrication, plagiarism, and double publication.

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