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Research Paper: The relationships between KCNQ1 promoter mutation and some cardiac disease risk factors with cardiac arrhythmia in the Bushehr population

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ABSTRACT

Objective: Many studies report that mutation in genes which encode the cardiac delayed rectifier potassium channel, such as KCNQ1, contributes to the long QT interval syndrome followed by cardiac arrhythmia.Goal of this case-control study is to explore the potential association between KCNQ1 gene promoter polymorphism and clinical Characteristics with cardiac arrhythmia in Bushehr population.

Methods: Clinical data, previous medical history, and blood samples were collected from 30 admitted patients in Bushehr hospitals with cardiac arrhythmia and matched healthy individuals as controls. The genetic variation of the promoter region of KCNQ1 was carried out by using single-strand conformational polymorphism (SSCP) analysis.

Results: NO mutation or polymorphism was identified in the promoter region of the KCNQ1 gene, but, the incidence rate of some cardiac disease risk factors, including diabetes mellitus, cigarette smoking, gender and age were significantly higher in the patients with cardiac arrhythmia.

Conclusions: The lack of mutation even in patients with a positive family history of cardiac arrhythmia revealed that mutation in the KCNQ1 gene might be not responsible for cardiac arrhythmia in the patients. But some clinical data, including diabetic mellitus, smoking habits, age and gender were the significant risk factors in patients with cardiac arrhythmia.

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1. Background

Cardiac arrhythmiaisthe common reasonof morbidity [1].Myocardial infarction (MI), ischemia and cardiomyopathy are the most structural abnormalities leading tolife-threated cardiac arrhythmias, however, not all ofthese structural abnormalities lead to arrhythmias [2]. Familial history of cardiac disease like MI, hypertension [3, 4] and also life-threatening or asymptomatic genetic background are some of the well-known risk factors involved in an individual's predisposition to cardiac arrhythmia [5]. Anothercommoncause of the cardiac event [6] and subsequent cardiac sudden deathislong QT syndrome(LQTS), which is illustratedby aprolonged QT interval on electrocardiogram and is able to predispose the person to cardiac arrhythmia like torsade-de-points and following sudden death by ventricular fibrillation.A number of mutations and polymorphism in genes which is encodingsome cardiac ion channels such as KCNQ1, KCNE1, and SCN5Ahave been involved in LQTS [7, 8]. KCNQ1 encodes a delayed rectifier potassium channel which outward potassium current of this channel is responsible for ventricular action potential plateau termination (myocardial repolarization) and reduction in its potassium current leads to prolongingthe action potential and so, long QT syndrome [9-11]. Around Seventy-five LQTS mutations were discovered in the KCNQ1gene [8]. Published data from the International LQTS Registry revealed that the frequency of cardiac events among subjects with LQT1 is about 63% [6]. Also many of non-genetic factors may influence on the cardiac disease and QT interval as well, including age, sex, hyperkalemia [12], weight, Body Mass Index, heart rate, blood pressure, total cholesterol [13-16], bloodglucose [17], coronary diseases [18] and some drugs [19], which all of these factors revealed a no significant [14-20] or significant association with the QT interval [21].It has been showed that Iran has a high risk of coronary heart disease [22].The highest rate of hypertension in Iran was detected in Bushehr province [23] and also the rate of cardiovascular risk factors such as obesity, smoking habits, and metabolic diseases was markedly higher in older women compared to older men in Bushehr province [24]. And also, the incidence rate of cigarette smoking among Bushehr's women above 25 years is high [25]. Since the rateof cardiovascular disease and KCNQ1 polymorphism in LQTS is moderatelyhigh, our objectives of this study were to (a) determine the incidence of KCNQ1 mutation or polymorphism among hospitalized patients with cardiac arrhythmiaassociated with a previous positive history of LQTS in Bushehr hospitals, and (b) Determine the possible correlation between KCNQ1 mutation or polymorphism and sex, age, diabetes, hypertension, andcigarettesmoking.Cytogenetic Location of KCNQ1 is 11p15.5-p15.4, which is located in chromosome 11 between positions 15.5 and 15.4 and molecular Location of KCNQ1 is base pairs 2,444,991 to 2,849,110 on chromosome 11 [26].

2. Methods

Study Subjects

This case-control study was carried out on 40 healthy individuals as control and 40Patients with cardiac arrhythmia and past positive history of prolonged QT interval referred to the cardiology department of Tamin Ejtemaee and Bentolhoda Hospitals in Bushehr (southern Iran) in the age range of 50-70 years old were randomly selected. Normal control individuals were randomly selected from the public population in Bushehr. Each person underwent a standardized interview to recognize past medical history, family history, and possible triggers for cardiac arrhythmia such as cigarette smoking, diabetic mellitus, age, gender, and hypertension.Matched age and ethnical subjects were chosen as controls. Control individuals have no positive history of cardiac arrhythmia or a prolonged QT interval. Genomic DNA extraction from peripheral blood lymphocyte was carried out by using the recommended protocols with the Blood Genomic DNA extraction mini kit (Favorgen Biotech). The study was approved by the guideline of the local ethics committee of the research council of the Bushehr University of Medical Sciences, and all individuals gave written informed agreement.

Next-generation sequencing for mutation detection in the promoter region of KCNQ1 gene

The promoter region of the KCNQ1 gene was

amplified by PCR method. We utilized standard protocol with the designed primers (using Oligo software 7.60) from the published KCNQ1 sequences in the NCBI database, Forward primer: 5'CCTTCCCCAGACGAGAGCA3',Reverse primer:5'TCCACCCATCCCAGCACAT3'. PCR was performed using recommended protocol with Favorgen Biotech.PCR mixture was prepared in a 20µL volume containing 200 pmol of each primer, 10 ng of genomic DNA, 2µLof10 × PCR buffer with 1.5mmolMgCl2,100 µmol deoxynucleotide triphosphates, and 1 unit of Taq DNA polymerase (Favorgen Biotech).The PCR was performed with the following program. Denaturation at 94°C for 30 seconds, annealing at 57 ºC for 30 seconds and extension at 72ºC for 3 seconds was done and repeated for 30 cycles.The final extension was done at 72ºC for 5 minutes. Then the DNA mixture cooled quickly on ice and kept for 5minutes. 6µL of each sample was loaded onto 6% nondenaturing polyacrylamide gels (acrylamide to bisacrylamide ratio = 40: 10) and electrophoresed at 90V for two hours at room temperature.The gel was stained with 0.5 µg/ml Ethidium bromide and visualized using a UV Gel documentation. In order to next-generation sequencing, we used Ion Torrent instruments (Life Technologies), and then the sequences were analyzed with Bio Edit Sequence Scanner Software.

Statistical Analysis

The $\chi 2$ test was used to determine whether the genotypic frequencies were in accordance with the accepted values based on the Hardy-Weinberg equilibrium (HWE) and then genotype frequencies between the cases and controls were compared to get odds ratios (ORs) with 95% confidence intervals (CIs). The SPSS statistical software (Chicago, IL, USA; version 18.0) was used for data analysis (Chi-square and OR were used to compare covariates between case and control samples). The multivariate logistic analysis included age, gender, blood pressure,diabetes status, previous family history, and smoking habits as covariates. P value<0.05 was significant, table [1].

3. Results

Clinical characteristics of the Study Population

A total of 40 patients with cardiac arrhythmia associated with a previous history of prolonged QT interval and 40 controls were subjected to

Table 1: The relationship between clinical findings and cardiac arrhythmia in the population of the studied.

Variable	Control, n=40	Case, n=40	Significancy
Age	60.7 ± 5.6	61.7 ± 4.5	
Gender (%Female)	35%	70%	*
Cigarette smoking (%)	40.00%	68%	*
Diabetes (%)	15%	35%	*
Family history	10%	18%	NS
Hypertension	37.50%	55.50%	NS
Age range of 60-70 years old (%)	50%	28%	*

*: significant with P<0.05.
NS: not significant.
The Age was defined as the age when the samples collected.

the study. The clinical and personal findings of these 40 patients and healthy subjects are in Table 1. The mean ± S.D. of age of the patients and controls were 61.7±4.5 and 60.7±5.6 years, respectively. Diabetic mellitus (RR, 3.05; 95% CI, 1.03 to 9.02; P=0.04), age range of 60-70 years old (RR, 0.38; 95% CI, 0.15 to 0.96; P=0.04), smoking habit (RR, 2.78; 95% CI, 1.13 to 6.89; P=0.03), and gender (RR, 0.35; 95% CI, 0.14 to 0.88; P=0.02) were strong risk factor for the cardiac arrhythmia occurrence, but previous family history (RR, 1.85; 95% CI, 0.49 to 6.93) and hypertension (RR, 2.41; 95% CI, 0.98 to 5.98) were not a significant risk factor for cardiac arrhythmia occurrence. Moreover, as it has been shown in figure 1, the incidence rate of cardiac arrhythmia is significantly higher in old age individuals, cigarette smoker, and women (P<0.05). There were no significant differences between the patients and control samples with regard to heredity and blood pressure status.

Identification of mutation or polymorphism in the KCNQ1 genome

To identify possible mutations or polymorphisms associated with cardiac arrhythmia and prolonged QT interval, the promoter region of

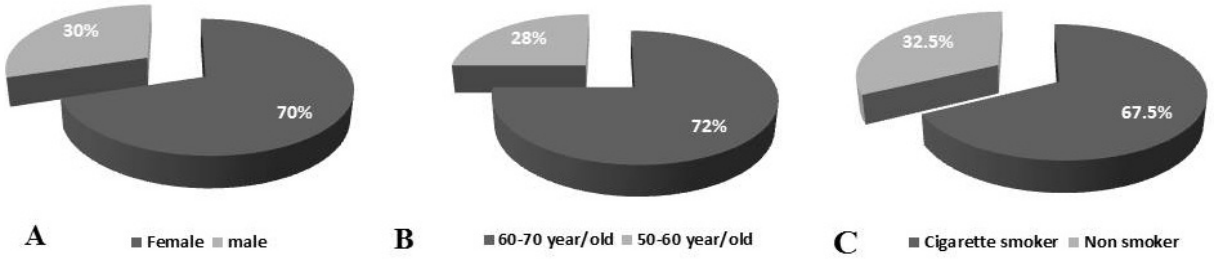

Figure 1: Effect of cardiac disease risk factors on the incidence rate of cardiac arrhythmia. The incidence rate of cardiac arrhythmia in women (**A**), old age patients **(B)**, cigarette smoker (**C**) and diabetes was significantly higher. P<0.05

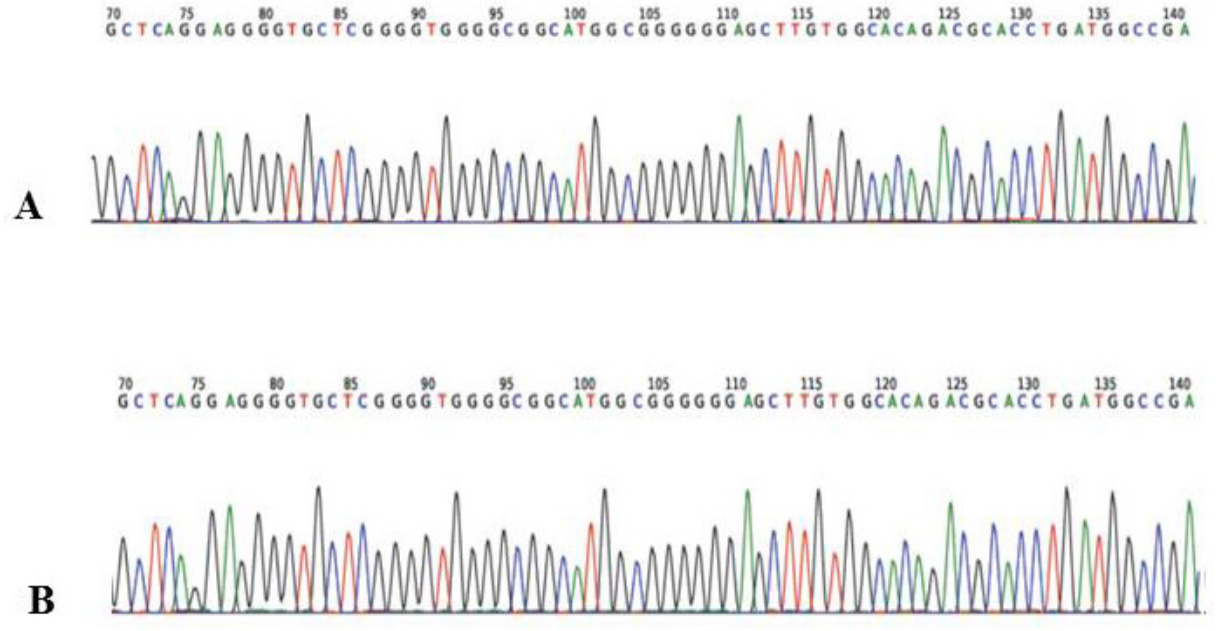

Figure 2: Representative figure of the KCNQ1 promoter sequencing. The sequence analysis of the PCR products did not show any nucleotide difference in both patients (A) and control individuals (B).

KCNQ1 was screened by using Bio Edit and Magna software. PCR products were sequenced to identify any mutation or polymorphisms. No mutation or polymorphism was detected in the KCNQ1 promoter region after sequence analysis. A representative portion of the sequencing has been shown in Figure 2.

4. Discussion

Despite an acceptable rationale for KCNQ1 as a responsible gene for LQTS followed by cardiac arrhythmia [7, 8], we could not identify any KCNQ1 mutations or polymorphisms in our study of 40 patients with cardiac arrhythmia associated with a positive history of the prolonged QT interval. It is well documented that other genes, like KCNE1 and SCN5A, are also involved in the development of prolonged QT syndrome [27], thus the lack of KCNQ1 mutation even in individuals with a positive family history of cardiac arrhythmia in this study, could indicate that mutation of other genes or non-genetic factors might be involved in the QT prolongation followed by cardiac arrythmia. On the other hand, since most of the patients (about 75%) did not have a positive family history of cardiac arrhythmia, and also no mutation was detected in these subjects, it can be concluded that non-genetic factors such as cardiac disease risk factors may have a role in cardiac arrhythmia. It was showed, that hypertension, tobacco consumption, diabetes status and family history were some of the associated factors for cardiac arrhythmia [4, 12]. Our results showed that diabetes was one of the considerable cardiac disease risk factor in patients with cardiac arrhythmia. Several studies in both types of diabetic patients (type I and II) have confirmed that there is a relation between prolonged QT interval duration and ischemic heart disease. They supposed that diabetic neuropathy may be involved in QT interval prolongation followed by cardiac arrythmai [17].Moreover, the prevalence rate of cardiac arrhythmia among old age, women and smoker patients was significantly higher in our study. This finding is in line with a cohort study in Bushehr which showed that the prevalence of the cardiac disease in the elder women was significantly higher than elder men [24]. Thus, a higher rate of cardiac arrhythmia in the elder women in this study is acceptable. Taken together, the risk factors such as diabetes, smoking habits, gender and age could be the reasons for prolonged QT interval and subsequent cardiac arrhythmia in the patients in our study. Further studies are necessary to investigate about mutations or polymorphism in other genes involved in the development of the LQT syndrome to obtain the effect of heredity on cardiac arrhythmia followed by the prolonged QT interval in the Bushehr population.

5. Conclusion

Although no mutation or polymorphism was detected in the study population, some clinical data, including diabetic mellitus, smoking habits, age and gender were the significant risk factors in patients with cardiac arrhythmia.

Ethical Considerations

Compliance with ethical guidelines

The protocol of this study was approved by the local ethics committee of the research council of the Islamic Azad University of Medical Sciences, Kazeron Branch. All samples were taken according to the guidelines provided by the ethics committee of the University, and informed consent was obtained from the participants.

Authors' Contribution

M.D. carried out the experiments and collected the data and revising. S. Kh. and F.S. contributed to results interpretation and A.B. contributed to data analysis, interpretation of the results, and manuscript writing and revising.

Conflict of Interests

The authors declare that they do not have any conflict of interests.

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