Efficacy of Long-term Selenium Supplementation in the Treatment of Chronic Keshan Disease with Congestive Heart Failure*

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Summary: Few effective treatments for chronic Keshan disease have been available till now. The efficacy of long-term selenium supplementation in the treatment of chronic Keshan disease with congestive heart failure is inconclusive. This study aimed to determine whether selenium supplementation is associated with a decreased risk of cardiac death in chronic Keshan disease with congestive heart failure by ten years of follow-up. A retrospective long-term follow-up analysis was performed on a monitored cohort consisting of 302 chronic Keshan disease patients with a mean age of 40.8±11.4 years. Of the 302 chronic Keshan disease patients, 170 (56.3%) were given selenium supplementation until the end point of follow-up. Cox proportional hazards regression models were used to identify the independent predictors of cardiac events. Our results showed that during the follow-up, there were 101 deaths of patients with chronic Keshan disease in the selenium supplementation group (101/170, 59.4%) and 98 in non-selenium supplementation group (98/132, 74.2%). Multivariate analyses suggested that selenium supplementation was associated with a decreased risk of cardiac death (HR 0.39, 95% CI 0.28–0.53) after adjustment for baseline age, sex, cigarette smoking, family history of Keshan disease, body mass index (BMI), heart rate, electrocardiogram (ECG) abnormalities, blood pressure, initial cardiothoracic ratio, left ventricular ejection fractions (LVEF) and whole-blood selenium concentration. Our ten-year follow-up analysis indicated that selenium supplementation, specifically combined with the use of angiotensin-converting enzyme inhibitor and beta blocker therapy, improved the survival of patients with chronic Keshan disease with congestive heart failure. BMI, selenium deficiency, male, combined ECG abnormalities, LVEF, and fast heart rate increased the risk of cardiac events.
Key words: Keshan disease; selenium supplementation; follow-up

Keshan disease (KD) is a fatal dilated endemic cardiomyopathy with unclear etiology, which has been prevalent in China for more than hundred years. The first epidemiological, clinical and pathological survey of KD was carried out in Keshan County, northeastern China, during winter 1935, when a severe outbreak that resembled a plague occurred[1]. Since then the disease has attracted a great deal of attention in China, and was named after the origin of first noticed appearance[2]. Later after the world war two, similar cases of KD were reported in Nagano Prefecture of Japan and in the northern mountains of North Korea[3].

KD patients are clinically classified into four categories (acute, subacute, chronic, and latent) based on the onset of attack, clinical features, and heart function[4–6]. In recent years, few cases of acute or subacute KD were reported, and latent KD and chronic KD are mainly present in KD endemic areas[7, 8]. Generally, chronic KD is manifested by congestive heart failure and varying degrees of pathological changes, and is characterized by severe cardiomyopathy. Multifocal myocardial necrosis is considered to be the

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*This work was supported by the National Natural Science Foundation of China (Nos. 81441016, 81273008).

main and the most characteristic feature of chronic KD. Up to now, only few effective treatment measures have been found for chronic KD, although some clinical trials have demonstrated that renin-angiotensin system blockers and beta blockers (BBs) reduce the mortality of chronic KD[9–12].

Oral selenium supplementation was reported to remarkably reduce the morbidity of KD in KD endemic areas, where the residents live with low selenium status[13–15]. There are few studies on the effect of long-term selenium supplementation in the treatment of chronic KD with congestive heart failure. The aim of the present study was to examine the efficacy of selenium supplementation for chronic KD with congestive heart failure by ten-year follow-up.

1 SUBJECTS AND METHODS

1.1 Study Population

We performed a retrospective survival analysis of chronic KD patients. All chronic KD patients were enrolled from monitored population in Huangling and Xunyi counties, Shaanxi Province, China, from April of 1995 to October of 2016, and all of them were new cases. They were diagnosed according to the National Criteria for Diagnosis of Keshan Disease (GB17020-1997)[16]. Chronic KD usually showed cardiac enlargement with moderate to severe chronic congestive heart failure (New York Heart Association Class II to IV). Patients received optimized therapy, including diuretics, digitalis, angiotensin-converting enzyme inhibitor (ACEI, captopril was mainly recommended and used), and BBs (mainly metoprolol tartrate), if patients had no medication contraindications and they could tolerate the drugs. All subjects with conditions such as cancer, diabetes mellitus, renal disorders, hepatocirrhosis, and age over 60 years old were excluded from the study. The present study protocol was approved by the Research Ethics Committee of Xi'an Jiaotong University, and all valid participants signed informed consents at each examination.

Medical records were created for all new cases, including medical history, a 12-lead electrocardiogram (ECG), chest radiography, and echocardiography. All the participants were examined at baseline. Additionally, above-mentioned examinations were carried out during follow-ups annually at late April, except echocardiography which was performed every two years. The participants were contacted by telephone during the remainder of the examination period. If a participant reported an interim hospitalization during the follow-up, then the hospital records were obtained and reviewed by a cardiologist.

1.2 Blood Samples

Fasting venous blood samples were collected. Pre-chilled EDTA-vials were used for measurements of whole blood selenium concentration and glutathione peroxidase (Gpx) activity.

1.3 Measurement of Whole Blood Selenium Concentration and Gpx Activity

Concentrations of selenium in the whole blood sample were assayed by a fluorometric method and hydride atomic fluorescence spectrometry (AFS2201A; Wantou Co., China). The whole blood Gpx activity was measured by a standard coupled spectrophotometric method using a micro-ultraviolet spectrophotometer (Nanjing Jincheng, China) at 412 nm. One enzyme unit of Gpx was defined as a decrease of 1 μmol/L concentration of glutathione in the reaction system per 8 μL whole blood reacted at 37ºC for 5 min after subtracting non-enzymatic reaction. Results were expressed as U/g Hb.

1.4 Follow-up and End Point

The primary end point of the study was cardiac events, including sudden cardiac death or cardiovascular mortality due to heart failure or cardiovascular thrombosis. Follow-up time was defined as the time from the baseline visit until the end point of cardiac event, or the end of the 10-year study for participants who did not have death event. Information on all-cause mortality was documented from hospital recordings or relatives' reports.

1.5 Statistical Analysis

Data analysis was performed using SPSS 13.0 software (IBM, USA). Continuous variables are presented as mean±standard deviation (SD). Comparisons between chronic KD groups were performed using chi-squared test for categorical variables and Student's *t*-test for continuous variables. A value of $P<0.05$ was regarded as statistically significant.

Univariate and Cox proportional-hazards regression analysis was performed to identify the independent predictors of cardiac events. The variables tested were age, sex, cigarette smoking, family history of KD, body mass index (BMI), heart rate, ECG abnormalities, blood pressure, initial cardiothoracic ratio, left ventricular ejection fractions (LVEF), and whole blood selenium concentration. The multivariate Cox regression model was built stepwise and based on the likelihood ratio testing of baseline characteristics.

Cumulative event rates for each group were obtained using Kaplan-Meier method for all the cardiac events, and compared with Wilcoxon log-rank test in all the patients with low to intermediate and high pre-test likelihood of cardiac events. Statistical significance was defined as $P<0.05$.

2 RESULTS

2.1 Characteristics of the Study Population

The deadline for the follow-up was November

2016. The final sample for the analyses consisted of 302 participants. The characteristics of the chronic KD patients are shown in table 1. At baseline, 206 (206/302, 68.2%) of the 302 chronic KD patients were women, and the mean age of all participants was 40.8±11.4 years. Of the 302 chronic KD patients, 170 (170/302, 56.3%) were given selenium supplementation until the end point of follow-up. A total of 199 (199/302, 65.9%) cases died during the follow-up (77.3±37.3 months). Of them, 101 (101/170, 59.4%) were in the selenium supplementation group, and 98 (98/132, 74.2%) in the group without selenium supplementation.

2.2 Correlation of Gpx Enzyme Activity with Selenium Blood Concentration

Whole blood selenium concentration at baseline ranged from 23.9 μg/L to 70.2 μg/L, and the mean value was 39.4±11.0 μg/L, which is much lower than that in Kłapcińska's report (80–250 μg/L) for the local residents in non-endemic areas of KD[17]. Whole blood selenium concentration (40.6±11.7 μg/L) in 170 chronic KD patients were significantly increased (63.4±6.8 μg/L) after six months of selenium supplementation (P<0.05). At baseline, the Gpx activity increased linearly with increasing blood selenium concentration (r=0.613, P<0.01; fig. 1).

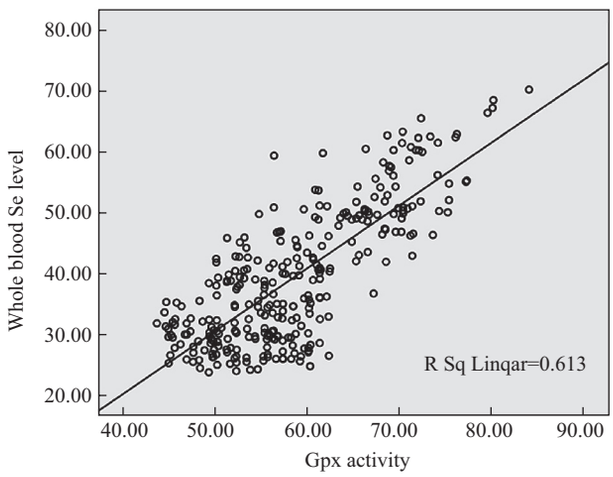

Fig. 1 Gpx activity and whole blood selenium (Se) concentrations in chronic KD patients
Scatter plot and regression results of correlation between Gpx activity and whole blood selenium concentration for all participants. Equation: Gpx activity = 35.04 + 0.596 Se level (r=0.613, P<0.01)

2.3 Treatment

The treatment was dominated by diuretics, which included spironolactone and hydrochlorothiazide (82.8%). Totally, 56.9% (n=172) of chronic KD patients were treated with digitalis. The combination of ACEI and BBs was used for treatment of 157 chronic KD patients (52.0%). 56.0% of all chronic KD patients (n=169) received selenium supplementation (sodium selenite tablet, 1 mg/week, which was distributed monthly by village doctors during the follow-up time), and 85 chronic KD patients also received ACEI+BBs among them.

2.4 Survival Data

The Kaplan-Meier estimates of cardiac events' cumulative hazard over time for chronic KD cases with selenium supplementation *vs.* without selenium supplementation showed a remarkable difference in cardiac events outcomes between the two groups (log-rank P<0.05, fig. 2). Low blood selenium levels at baseline were associated with an increased risk of cardiac events.

Selenium supplementation and ACEI+BBs during the follow-up were associated with the decreased risk of cardiac death (for selenium supplementation: HR 0.39, 95% CI 0.28–0.53; for ACEI+BBs: HR 0.57, 95% CI 0.39–0.84) after adjustment for baseline age, sex, cigarette smoking, family history of KD, BMI, heart rate, ECG abnormalities, blood pressure, initial cardiothoracic ratio, LVEF, and whole blood selenium concentration (table 2).

3 DISCUSSION

The results of the present study showed that the selenium supplementation to chronic KD patients

Table 1 Baseline characteristics of the study population

Characteristics	All (n=302)	Se supplement (n=170)	No Se supplement (n=132)	P value
Age (year)	40.8±11.4	38.5±11.5	43.8±10.5	<0.05
Women (n, %)	206 (68.2)	120 (70.6)	86 (65.2)	0.32
Family history of KD (n, %)	72 (23.8)	49 (28.8)	23 (17.4)	<0.05
Combined ECG abnormalities (n, %)	111(36.8)	49 (28.8)	62 (47.0)	<0.01
Initial cardiothoracic ratio	0.58±0.03	0.58±0.03	0.57±0.03	0.51
Whole blood Se level (μg/L)	39.4±11.0	40.6±11.7	37.9±9.8	<0.05
Smoker (n, %)	58 (19.2)	31 (18.2)	27 (20.5)	0.66
Heart rate (beat/min)	78.3±4.2	78.3±4.9	78.3±3.1	0.96
Body mass index (kg/m^2)	20.9±1.6	20.8±1.6	21.0±1.5	0.36
Systolic blood pressure (mmHg)	118.6±10.4	116.7±10.6	120.3±9.6	0.20
Diastolic blood pressure (mmHg)	76.7±6.2	74.9±5.9	78.2±6.1	0.08
LVEF (%)	32.2±3.7	32.0±3.9	32.4±3.5	0.34

ECG: electrocardiogram; KD: Keshan disease; SD: standard deviation; Se: selenium; LVEF: left ventricular ejection fraction

Table 2 Hazard ratios for mortality in chronic KD patients

Characteristics	Wald	*P*	Exp (β)	95% CI
Gender	12.84	<0.01	0.42	0.26–0.68
Combined ECG abnormalities	4.81	<0.05	1.48	1.04–2.08
LVEF (%)	89.92	<0.01	0.76	0.72–0.81
Heart rate (beat/min)	14.11	<0.01	1.08	1.04–1.12
Whole blood Se level	15.57	<0.01	0.95	0.93–0.97
BMI	11.15	<0.01	1.12	1.08–1.36
Se supplementation	34.29	<0.01	0.39	0.28–0.53
ACEI + BB	8.33	<0.01	0.57	0.39–0.84

ECG: electrocardiogram; LVEF: left ventricular ejection fraction; Se: selenium; BMI: body mass index; ACEI: angiotensin converting enzyme inhibitor; BB: beta blocker; CI: confidence interval

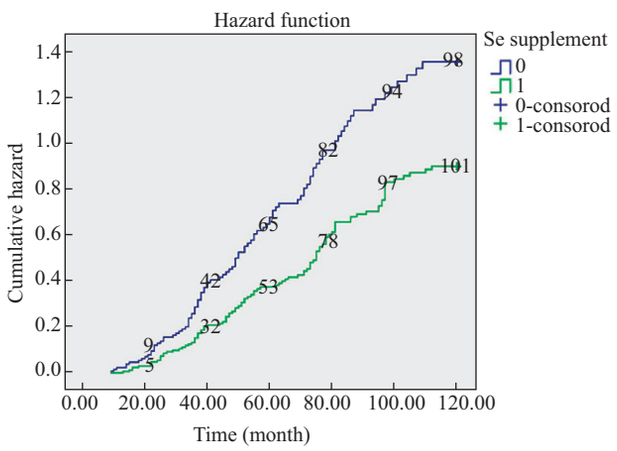

Fig. 2 Kaplan-Meier estimates of cardiac events' cumulative hazard over time of selenium supplement *vs.* no selenium supplementation
Se: selenium

was associated with a lower risk of developing cardiac death during the 10 years of follow-up. Several previous studies have reported that selenium supplementation can improve the survival rate of chronic KD patients[18, 19]. However, to our knowledge, long-term follow-up of the effects of selenium supplementation on patients with chronic KD has not been available yet.

The etiology of KD has not been determined until now, although multiple hypotheses have been suggested, including intoxication with environmental toxicants or mycotoxins, viral infection, and nutrition deficiency caused by a monotonous diet lacking minerals and vitamins, such as magnesium, molybdenum, or thiamin[20–23]. Among the multitude of potential etiologies for KD, selenium-deficiency hypothesis has been considered as the most convincing one[24]. KD is documented to be closely associated with selenium-deficiency, and related with reduced activities of selenium-dependent antioxidant enzymes, such as Gpx. Some studies have indicated that selenium-deficiency can cause myocardial cell injury and apoptosis in rats[25, 26]. In this research, lower levels of selenium were correlated with a decrease in GPx activity, and were associated with the progression of chronic KD to cardiac events (HR 0.95, 95% CI 0.93–0.97).

Large-scale trials have revealed that a combined therapy of ACEI and BBs had a pronounced effect on left ventricular (LV) function and improved long-term survival of patients with congestive heart failure[27–30]. Earlier reports have further indicated that ACEI and BBs had a significant effect on LV function and five-year survival of chronic KD patients with congestive heart failure[10]. A recent study reported a trend of better survival rate of chronic KD patients with selenium supplementation in comparison to the control group without selenium supplementation, although the difference was not statistically significant[18]. The lack of significance may be related with the limitations of short follow-up periods (9 months) and small sample size (105 cases)[18].

In the present study, 85 chronic KD patients in the ACEI+BBs group with selenium supplementation had higher 10-year survival rate than those in the ACEI+BBs group without selenium supplementation [57.6% (49/85) *vs.* 33.3% (24/72), log-rank $P<0.05$], and the 10-year survival rate in the selenium supplementation group without ACEI+BBs was also higher than that in the group receiving neither selenium supplementation nor ACEI+BBs [23.8% (20/84) *vs.* 16.4%(10/61), log-rank $P<0.05$]. After adjustment for baseline age, sex, cigarette smoking, family history of KD, BMI, heart rate, ECG abnormalities, blood pressure, initial cardiothoracic ratio, LVEF, and whole blood selenium concentration, the combination therapy of selenium supplementation and ACEI+BBs was found to have an obvious positive effect on survival rate during the 10-year follow-up (for ACEI+BBs and selenium supplementation: HR 0.71, 95% CI 0.60–0.83; for ACEI+BBs: HR 0.57, 95% CI 0.39–0.84; selenium supplementation: HR 0.39, 95% CI 0.28–0.53).

KD is most prevalent in rural areas in China, most of which are mountainous, remote districts and have inconvenient traffic. Most KD patients are poor and disadvantaged in the utilization of health services. Therefore, cheap and effective treatment is very important for them. Drugs, such captopril and

metoprolol tartrate, are cheap enough in China for chronic KD patients to afford. Also in this study, we showed that captopril and metoprolol tartrate combined with selenium supplementation was more cost-effective than the medication of captopril and metoprolol tartrate together. This finding is worth popularization and application in the therapy of KD patients.

Nevertheless, there are some limitations in the present study. It is a single-center study in Shaanxi province, north of China. Therefore, the results may not be applicable to chronic KD patients in southern parts of China. The sample size was relatively small and, as a result, certain trends did not reach the statistical significance, thus lowering the statistical power. Also the death cause of some chronic KD patients was not certified by a coroner, especially for those who died at home due to local customs. However, sudden death witnessed by bystanders and the fast aid of paramedics and/or general practitioners gave a diagnosis in almost all cases.

In conclusion, the present 10-year follow-up demonstrated that selenium supplementation, specifically combined with the use of ACEI+BBs therapy, was associated with better survival of KD patients; BMI, selenium deficiency, male, combined ECG abnormalities, low LVEF, and increased heart rate elevated the risk of cardiac events.

Conflict of Interest Statement

The authors declare that they have no competing interests in this study.

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(Received May 21, 2018; revised Oct. 29, 2018)