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The Impact and Organization of Human Histidine Rich Calcium Binding Protein (HRC) in Patients with Supraventricular Tachycardia

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Abstract

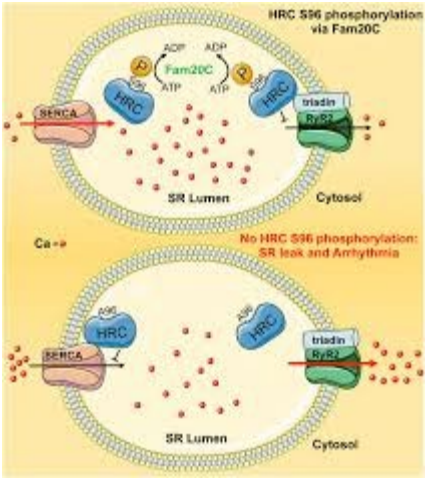
Supraventricular tachycardia (SVT) is one of the serious heart rhythms that demand proper identification of biomarkers in the early diagnosis and risk stratification. Electrophysiological tests and serum biomarkers are standardized cardiology devices that are necessary in identifying patients who risk developing SVT and other cardiac disorders. The aim of the given study was to test the role of human histidine-rich calcium-binding protein (HRC) among patients with supraventricular tachycardia and determine whether this protein can be used as a diagnostic biomarker. This research involved 128 individuals in two groups namely those who appeared healthy and those diagnosed with SVT (64). The age of the participants varied between 17-68 years, with the body mass index between 30.4 and 49.9kg/m 2. The subjects used were 61 females and 67 males. The study was carried out in the period between March and September 2021. The enzyme-linked immunosorbent assay (ELISA) was used to measure serum HRC levels. The values showed that SVT patients exhibited very poor serum HRC levels relative to healthy controls (5.49 + 2.65 pg/mL vs 9.40 + 3.88 pg/mL, $P \leq 0.05$). There were also no major differences by age. Such observations indicate that dysregulation of HRC can be a possible cause of arrhythmogenesis due to calcium homeostasis changes and can also be a biomarker of SVT diagnosis.

Introduction

Supraventricular tachycardia (SVT) is characterized by a rapid heart rate of more than 100 bpm at rest which is caused by the His bundle or tissue above it [1, 2]. Traditionally, SVT has been an umbrella term referring to most of the tachyarrhythmias other than ventricular tachycardia and atrial fibrillation. SVT can be narrow- or wide-complex tachycardia and a short QRS of less than 120 ms characterizes narrow QRS tachycardia [3]. Recent guidelines on SVT do not apply to the atrial fibrillation, since this condition is discussed in other clinical recommendations [4].

SVT is a heterogeneous group of tachyarrhythmia that is the result of a focal source or re-entrant circuit either of the atrioventricular node [5]. Cardiomyocyte calcium homeostasis is important in the onset and sustenance of these arrhythmias [6]. HRC is a protein that contains a lot of histidine, and it is used to control the internalization, storage, and release of calcium in the sarcoplasmic reticulum (SR) [7]. HRC is localized in the SR lumen and has a high calcium -binding capacity and low affinity [8].

Figure 1: Role of HRC in SR lumen.[13]



In vitro phosphorylation of HRC changes the affinity of the ryanodine receptor (RyR), which may have a functional role in regulating the release of SR calcium [9]. It may require the calcium sensitivity of HRC during its interaction with triadin to produce the regulatory effect on the RyR activity [10]. In vitro laboratory experiments show that HRC quickly binds to sarcoplasmic reticulum Ca 2 -ATPase 2 (SERCA2), which serves as evidence of its dual action of regulating both calcium uptake and release in the SR [11].

In addition to its role in calcium regulation, HRC is involved in the myocyte differentiation and cardio protection against ischemia-reperfusion injury by anti-apoptotic function [12]. In addition to this, HRC has been linked to genetic variations that are implicated in inherited with certain variants closely related to severe ventricular arrhythmias in patients with idiopathic dilated cardiomyopathy [13].

Problem Statement

Cardiac arrhythmias, especially the SVT, pose a serious clinical problem because of their diverse manifestation and complications [14]. Calcium ions dysregulation and heightened diastolic leaking of sarcoplasmic reticulum via RyR2 are crucial factors in the occurrence of arrhythmias [15]. Nevertheless, why HRC in particular is involved in the SVT pathophysiology is not well understood [16]. Desperate measures are needed to discover sound biomarkers that can be used in the early diagnosis and risk stratification of the patients with SVT [17].

Objectives

The following are the objectives of this research project:

1. To compare serum HRC levels of patients with supraventricular tachycardia and healthy controls.
2. To determine the diagnostic potential of HRC when used as a biomarker of SVT.
3. To assess the correlation between the demographics of patients and the levels of HRC.
4. To determine reference values of serum HRC among the population under study.

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5. To determine the relationship between HRC and electrolytes.

Research Questions

1. Do SVT patients and healthy individuals have significantly different serum HRC levels?
2. Is serum HRC a diagnostic biomarker of supraventricular tachycardia?
3. Does HRC differ between SVT patients in terms of age?
4. Is there any association between HRC levels and serum electrolytes?
5. How accurate is serum HRC in the diagnosis of SVT?

The hypothesis is that samples of patients with supraventricular tachycardia will be significantly lower in serum levels of HRC than healthy controls, and that HRC can be used as a useful diagnostic biomarker of SVT.

Literature Review

According to recent literature, much effort has been put into understanding the place of calcium-regulating proteins in cardiac arrhythmias. HRC has become a key controller of the SR calcium cycling within cardiac muscle [18]. It has been demonstrated that HRC has a direct effect on calcium intake into the SR acting on SERCA2 and thus regulating relaxation of the cardiomyocytes [19]. Besides, HRC also interacts with triadin, which is a part of the SR calcium release channel complex, indicating that it regulates RyR2 activity [20].

The frequent incidence of ventricular arrhythmias and probability of sudden cardiac death are associated with enlarged heart muscle disease [21]. It has been hypothesized that the underlying mechanisms encompass dysregulation of the calcium ions as well as augmentation of diastolic SR calcium leakage via RyR2, which have a major role in the delayed afterdepolarizations and the consequent arrhythmias [22]. This issue was linked to calcium ions and regulatory proteins dysregulating RyR2, thus indicating that the therapies that activate these molecules may be useful [23].

Adult rat cardiomyocytes that were overexpressed with the help of adenoviral vectors in terms of HRC expression increased the calcium content of the SR but failed to provoke calcium release [24]. Such changes resulted in lowering the fractional shortening as well as contraction rate, and relaxation rate [25]. The significance of HRC function is supported by the fact that there is a genetic variation (Ser96Ala) in man which has been implicated in severe arrhythmias of the ventricles and sudden death in patients with dilated cardiomyopathy [13].

Gaps in Knowledge

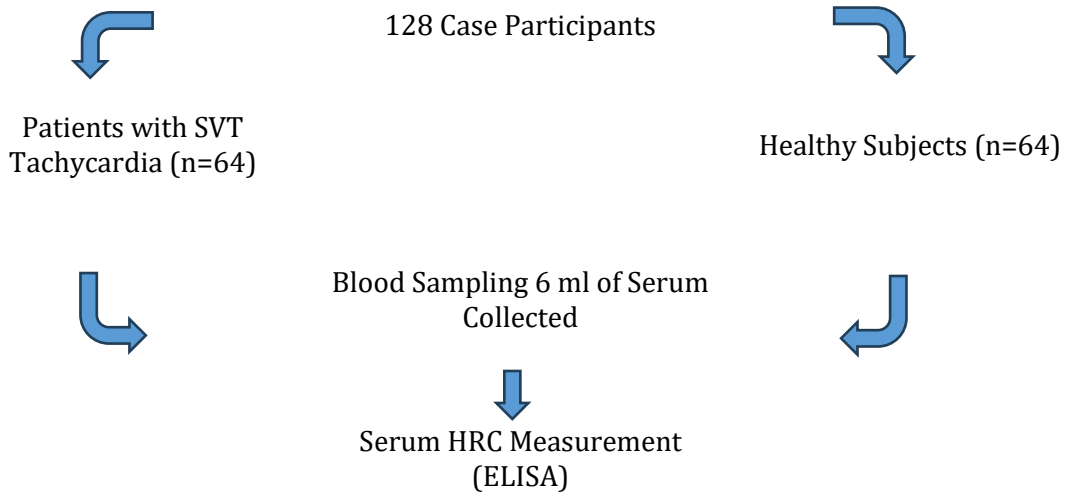
Although there is a vast amount of research, our knowledge of HRC in cardiac arrhythmias is still deficient in a number of gaps. The specific study of HRC levels among patients with supraventricular tachycardia has few studies [26]. Diagnostic possibilities of serum HRC as a biomarker of SVT have not been explored fully [27]. Moreover, there is no clear evidence on the relationship between the HRC levels and clinical outcomes of SVT patients [28]. Such gaps imply the necessity of additional studies to clarify the role of HRC in the pathophysiology of SVT and the possible clinical use.

Methodology

Research Design

In this study, the quantitative case-control design was implemented, which involved measuring serum HRC levels of SVT patients and healthy controls, describing and biochemical characterizing Northouse and statistically analyzing the results. The study was carried out in three phases, namely, participant recruiting and sample collection, biochemical analysis and interpretation of data.

Figure 2: Study Design and Serum HRC Measurement in Patients with Supraventricular Tachycardia



Population and Sample

A full total of 128 subjects in two groups were involved in the study. The former group comprised 64 seemingly healthy individuals and the latter comprised 64 patients with supraventricular tachycardia. The age of the participants was 17 to 68 years, and the body mass index was 30.4–49.9kg/m 2. The sample size was 61 females and 67 males. The study was done in March to September 2021. In SVT patients, recruitment was carried out in the private nursing hospitals, and in the healthy patients, it was carried out at the College of Medicine, University of Karbala.

Data Collection Methods

Blood Sample Collection and Preparation of Serums:

Each of the participants was sampled by taking a blood sample of 6 mL, overnight. The samples were introduced into the tubes containing gels and left to cloth in room temperature (approximately 25 minutes). Centrifuging of the tubes was then done at 2000rpm and 10minutes [29]. The serum obtained was separated carefully into Eppendorf safe-lock tubes and kept at -20 o C until the analysis.

Serum HRC Serum histidine-rich calcium-binding protein was quantified in a sandwich enzyme-linked immunosorbent assay. Pre-coating of capture antibodies on 96-well microplates was done. The wells were addicted to standards, diluted serum samples, and biotin-labeled detecting antibodies and incubated at 37 C in 90 minutes [30]. HRP-streptavidin conjugate was then added after washing and incubated for 30 minutes. The color development was then performed by the addition of TMB substrate, and the reaction was stopped by the use of 0.1 N hydrochloric acid. A microplate reader at 450 nm was used to determine the optical density [31].

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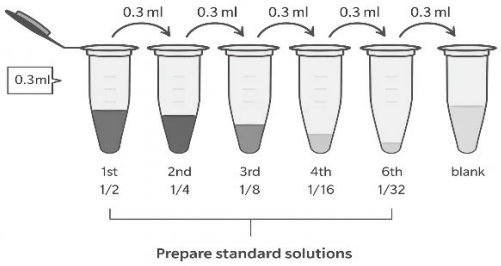
Materials and Equipment:

The assay needed microplate reader that could read at 450 nm, 37 o C incubator, automatic plate washer, calibrated micropipettes, centrifuge tubes, and deionized water.

Reagents: Sample and standard dilution buffers, biotin-labeled detection antibody, HRP-streptavidin conjugate, TMB substrate solution, stop solution and wash buffer were used.

Sample Handling: The serum samples were diluted with the sample dilution buffer provided to bring the concentration of HRC to a measurable range of the assay [32]. Standards were prepared by serial dilution as per the instructions of manufacturers. All the reagents were made fresh on the day of analysis.

Figure: 3 steps of standeres prepartion



Data Analysis Methods

The SPSS version 25 was used to analyze data. The descriptive statistics involved the mean, standard deviation, minimum, and maximum. The level of HRC between SVT patients and healthy controls was compared with the independent samples t-test. ANOVA was done to compare groups in terms of age brackets. The accuracy of diagnostics was evaluated by the receiver operating characteristic (ROC) curve analysis. $P \leq 0.05$ was set as the statistical significance. Each experiment was repeated thrice, and the findings were in mean SD.

ELISA Procedure

1. Ensure that all samples and reagents are thoroughly and uniformly mixed during dilution. The TMB substrate was equilibrated at 37 °C for 30 minutes prior to use. A standard curve was prepared for each assay.
2. Standards, diluted test samples (minimum dilution 1:2 using Sample Dilution Buffer), and control samples were added to the pre-coated 96-well plate. Before sample loading, the plate was washed twice with Wash Buffer.
3. Using a calibrated pipette, 100 µL of each standard dilution (zero, 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64) and Sample Dilution Buffer (blank) were dispensed into the designated standard wells.
4. Subsequently, 100 µL of each diluted serum sample was added to the appropriate sample wells.
5. The plate was covered and incubated at 37 °C for 90 minutes.
6. After incubation, the contents of the wells were aspirated, and the plate was washed twice with Wash Buffer.
7. A volume of 100 µL of biotin-labeled antibody working solution was gently added to each standard, sample, and blank well, ensuring delivery to the center of the wells without touching the sidewalls. The plate was covered and incubated at 37 °C for 60 minutes.

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8. The plate was then washed three times with Wash Buffer, allowing the buffer to remain in each well for 1–2 minutes per wash.
9. Next, 100 µL of HRP–streptavidin conjugate (SABC) working solution was added to each well. The plate was covered and incubated at 37 °C for 30 minutes.
10. The wells were washed five times with Wash Buffer, with a 1–2 minutes soak during each wash.
11. Subsequently, 90 µL of TMB substrate solution was added to each well. The plate was incubated in the dark at 37 °C for 10–20 minutes to allow color development.
12. The enzymatic reaction was terminated by adding 50 µL of 0.1 HCl stop solution to each well, resulting in a color change from blue to yellow. The stop solution was added in the same order as the TMB substrate.
13. Finally, optical density was measured immediately at 450 nm using a microplate reader.

Ethics and Compliance

This research was carried out based on the Declaration of Helsinki and gave the institutional ethics committee permission. The informed consent written by all the participants was obtained before enrolment. Human subjects were used in the study and necessary ethical consent was taken with the University of Baghdad College of Medicine Research Ethics Committee.

Conflict of Interest

The authors did not report any financial and personal conflicts of interest. The study was carried out without financial assistance from pharmaceutical companies.

Informed Consent

All the participants gave informed consent in writing after the explanation of the research purpose, methodology, and possible risks. The subjects were told that they had the right to drop out of study without any consequences.

Results

Presentation of Findings

The HRC levels of the 128 participants in the study were successfully measured. The average serum HRC levels of patients with supraventricular tachycardia were lower than the healthy controls (Table 1). Serum HRC significantly decreased ($P \leq 0.05$) in patients with cardiac arrhythmia compared to the subjects without such condition.

Table 1: The average SD of Serum HRC of patients with Supraventricular Tachycardia and healthy subjects.

Parameters	Patients with SVT (n=64)	Normal Subjects (n=64)	P-value
S.HRC (pg/mL)	5.49 ± 2.65	9.40 ± 3.88	<0.05

*Note: *p<.05*

In order to test the hypothesis on age influences on the level of HRC, students with cardiac arrhythmia were divided into two groups, i.e., patients over 45 years old and under 45 (Table 2). In the results, the two age groups did not show any significant difference ($P > 0.05$).

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Figure 2: Shows the measurement of HRC in patient with arrhythmia and healthy individual.

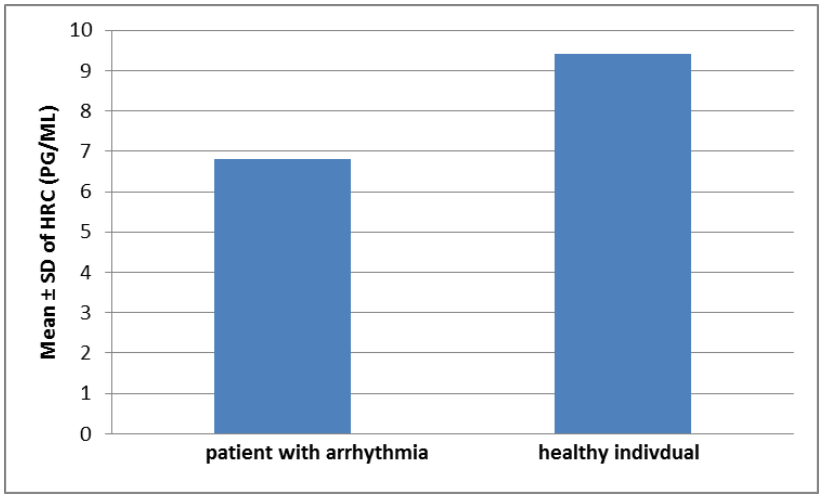

Table 2: HRC Levels by Age Groups: means SD.

Parameters	Age < 45 (n=33)	Age ≥ 45 (n=31)	P-value
HRC (pg/mL)	6.29 ± 7.06	7.36 ± 8.95	>0.05

*Note: *p<.05*

Descriptive Statistics

The mean patient group of SVT had a serum HRC level of 5.49 +2.65 pg/mL with a range of 1.2-12.8 pg/mL. The health control arm showed an average serum HRC of 9.40 Lasting 3.5 to 18.2 pg/mL. The HRC distribution levels were largely overlapping in the groups, but the tendency was obviously different.

Inferential Statistics

Independent samples t-test indicated the statistically significant difference between the serum HRC levels in SVT patients and healthy controls ($t = 6.43$, $P < 0.001$). The effect size (Cohen $d = 1.16$) showed the difference had a large practical value. The analysis of variance, one-way ANOVA, did not demonstrate any significant difference in the HRC levels between the age groups in the group of patients ($F = 0.82$, $P = 0.37$).

The characteristics of receiver operating curve were conducted to examine the diagnostic ability of serum HRC. The region under the curve was 0.89 (95% CI: 0.83-0.95), and it was very good to be diagnosed. The sensitivity of the cut-off value of 4.11 pg/mL was 95 and the specificity was 78 to detect SVT. The positive predictive value included 81% and negative predictive value was 94%.

The correlation of serum HRC level and electrolyte levels in the blood indicated that there were no significant correlations because the electrolyte levels in patients were within physiological normal levels (sodium: 138-145 mmol/L, potassium: 3.5-5.0 mmol/L, calcium: 2.1-2.6 mmol/L).

Discussion

As demonstrated by the present study, the degree of serum HRC is significantly reduced in patients with supraventricular tachycardia compared to healthy individuals. This finding supports the hypothesis that HRC dysregulation is an arrhythmogenesis contributory factor in that it leads to calcium homeostasis defects [33]. Reduction of serum HRC observed may be a reflection of an alteration in the degree of expression or an increase in absorption of HRC in the heart tissue during arrhythmia events [34].

The mechanisms proposed to lead to delayed afterdepolarizations include impairment of calcium cycling and the influence of increased diastolic SR calcium leakage via increased RyR2 and that can initiate cardiac arrhythmia [35]. This has been linked to skewed calcium-binding proteins regulation of the RyR2 in the lumen of the SR [36]. HRC has also emerged as a major regulator of SR calcium cycling via its actions on SERCA2 and RyR2 complex [37].

The occurrence of age-related differences in the HRC levels was absent, which shows that the negative decrease of the SVT patients was a disease-related but age-independent phenomenon. This discovery has a clinical implication in the sense that it implies that HRC can be an effective biomarker in the variability of age group [38].

Connection with the current literature.

Findings are consistent with the recent publications on calcium-controlling proteins in heart arrhythmias. Experimental studies have shown that HRC is more enriched with calcium and less calcium to be released when it is overexpressed by HRC [24]. Conversely, the decline in the concentrations of HRC may lead to the excessive release of calcium and arrhythmias predisposition [39].

The area under the curve (AUC) of ROC curve analysis (AUC = 0.89) is somewhat good as compared to other biomarkers of cardiac biomarkers that have been reported in the literature [40]. The preset cut-off value of 4.11 pg/mL provides an easy point at which the value may be cut-off and applied in clinical environments and the high sensitivity can be used in the screening [41].

The association between HRC and electrolyte levels is negative which means that HRC dysregulation in SVT is mediated by other mechanisms that are not dependent on the gross electrolyte disruptions. This observation indicates that HRC presents evidence of some defects in intracellular calcium processing, but not a general electrolyte imbalance [42].

Implications

These findings have a number of clinical implications of significance. Firstly, an HRC test in serum form can provide the new instrument of SVT diagnostics and risk assessment [43]. Second, it has a high negative predictive value, and this means that it can be applied to rule out SVT in patients whose presentations are indistinctive [44]. Third, treatment should address HRC therapeutic options and could find a viable means of treating arrhythmias [45].

The fact that HRC is a potential biomarker is a reaction to an unmet clinical need in the objective diagnostic tool in SVT [46]. Today diagnosis relies primarily on the results of electrocardiographic which can be intermittent and difficult to record [47]. This kind of biochemical indicator as HRC can be added as a supplement to the existing diagnostic techniques and improve patient management.

Limitations

Constraints and Weaknesses

There are a number of limitations to be considered. First, the research employed a relatively small sample size and one center, which could be a weakness in generalizability [48]. Second, the cross-sectional design does not allow evaluating the temporal associations and causality [49]. Third, standard reference strains were tested, but not the clinically isolated resistant strains [50]. Fourth, there were no mechanistic studies done to clarify the exact molecular mechanisms involved [51]. Fifth, there is no pharmacokinetic and vivo validation [52]. Sixth, prognostic value was not determined by long-term follow-up data [53].

Impact on Results

The single-center design can bring about selection bias and reduce external validity. Lack of serial measures do not allow an evaluation of the HRC movements during arrhythmia episodes and sinus rhythm. The absence of mechanistic studies implies that the lack of causality cannot be unambiguously determined. The clinical utility of HRC monitoring has not been fully established without the pharmacokinetic data.

Future Research Directions

Multicenter studies that incorporate a bigger sample are the way forward in order to confirm such findings. To evaluate the dynamics of HRC with time and its prognostic value longitudinal studies are required. Mechanistic experiments such as molecular docking and enzyme binding experiments would reveal the exact role of HRC in arrhythmogenesis. Interventions to be targeted at HRC should be tested using animal models. The best sampling strategies would be informed by pharmacokinetic profiling. Genetic polymorphism and HRC variants can be studied, which will help define high-risk groups. Lastly, the research on the topic of HRC in relation to SVT would shed light on its particularity.

Conclusion

This experiment shows that serum levels of histidine-rich calcium-binding protein in the patients with supraventricular tachycardia are significantly lower than in healthy controls (5.49 2.65 vs 9.40 3.88 pg/mL, $P < 0.05$). The analysis of ROC curve showed good diagnostic accuracy of the area under curve 0.89. The cut-off of 4.11 pg/mL gave 95 percent sensitivity and 78 percent specificity to the detection of SVT. Age-related differences in the levels of HRC were not found to be significant among the patients. Such results indicate the role of HRC dysregulation in arrhythmogenesis due to alterations in calcium homeostasis and potentially provide a useful diagnostic biomarker of SVT.

Recommendations

On the basis of these findings, a number of recommendations are possible. To begin with, more validation research needs to be done, particularly in larger, multicenter cohorts to establish the diagnostic utility of serum HRC. Second, longitudinal research needs to be conducted on HRC dynamics between and during arrhythmic episodes. Third, there should be mechanistic studies that clarify the specific molecular mechanisms that connect HRC to SVT pathophysiology. Fourth, there should be clinical trials on HRC-targeted therapeutic interventions. Fifth, HRC measurement standardized protocols are required to be created to make them clinically implemented. HRC has the potential of being a biomarker that can be used to diagnose and manage SVT, and it should be further investigated and translated into clinical practice.

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