Plasma Na\(^+\), K\(^+\)-ATPase Activity
In Some Cardiovascular Diseases in Elderly

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ABSTRACT

Cardiovascular diseases, (CVD), are the major cause of early mortality, accounting for almost 25% of deaths globally. CVD events are the net result of integration of several risk factors namely: aging, obesity, hypercholesterolemia, hypertension, diabetes mellitus, smoking and lifestyle.

Saudi Arabia has gone through significant urbanization over the last 40 years with significant improvement in social and economical status. This has led to change in lifestyle, accommodating more of the Western habits with respect to diet and physical activity. These factors have either led or augmented other risk factors of CVD.

The aim of this study is to provide a helpful test that could be used for the early diagnosis and prognosis of CVD by advancing age through studying plasma activity of the enzyme Na\(^+\), K\(^+\)-ATPase in different classes of CVD such as valvular heart disease, ischemic heart disease, arrhythmia and heart failure in relation to other biochemical parameters as plasma glucose, urea, creatinine, triglyceride, cholesterol, HDL, LDL, Na, K, Mg, Ca, Cl and P besides the relevant plasma enzymes as CK, LDH, AST, ALT compared to a matched normal control group.

A total of 95 blood samples were collected from patients of different CVD, in addition to 20 blood samples from normal controls. The age for both groups ranged between 50 and 85 years of both sexes, (78 females and 37 males).

The results point to a statistically significant increase in Na\(^+\), K\(^+\)-ATPase activity in most of CVD patients groups under study. Other biochemical parameters showed almost the well known clinically agreed upon pattern. It could be concluded that Na\(^+\), K\(^+\)-ATPase should be added as a predictor test for the diagnostic and prognosis of cardiovascular diseases.

INTRODUCTION

Aging is defined as the regression of physiological function with advancement of age. In the heart there is increased fat, collagen and elastic tissue. In people over 75 years old, heart failure occurs ten times more often than in younger adults. CVD, affect 50% of those over the age of 65 and is a leading cause of death. Mortality from heart disease has declined significantly in the United States over the last three decades, probably due to improved medical treatment and the active information campaigns to educate people about smoking, hypertension and hypercholesterolemia which have
been recognized as the three major risk factors of CVD.

It has been found that activity of erythrocyte sodium pump declines with aging in rabbits, as a result of a reduction in the number of Na⁺, K⁺-ATPase units(1). A positive correlation between urinary Na⁺/ K⁺ ratio and Na⁺, K⁺-ATPase activity of erythrocyte membrane was found in human female adolescents either with normal blood pressure or with a family history of hypertension, suggesting significant correlations in terms of the abnormal membrane cation fluxes associated with essential hypertension(2).

The activity of cardiac Na⁺, K⁺-ATPase from young guinea pigs is significantly higher than those from the older. Activity of Na⁺, K⁺-ATPase in young tissue preparations was less inhibited by ouabain than in older tissue preparations. These differences may be related to the observations that young animals and humans are less sensitive to cardiac glycosides than adults(3).

The age-dependent change in myocardial Na⁺, K⁺-ATPase concentration could be ascribed to variation in the ratio between the amount of Na⁺, K⁺-ATPase and muscle mass during development. Since myocardial Na⁺, K⁺-ATPase is the receptor for cardiac glycosides, this may in part explain the situation in the young age(4).

Na⁺, K⁺-ATPase is essential for excitability and contractility of muscle tissue. The decrease in concentration of the pump in skeletal muscle may contribute to the limitation of exercise capacity in congestive heart failure(5). At the same time it was found that Na⁺, K⁺-ATPase activity was significantly reduced in the untreated hypertensive patients when compared with normal controls(6)(7)

**AIM OF WORK**

In Saudi Arabian population aged 30-64 years old the prevalence of risk factors for CVD such as obesity, diabetes mellitus, hypercholesterolemia and other cholesterol related risk factors grows high(7).

In all countries of the Region, there is increasing awareness about CVD as a main cause of morbidity and mortality. A survey preformed in the year 2000 in Saudi Arabia showed the increasing number of elderly in Saudi Arabia and hence the increased number of cardiovascular patients, which is a good reason to investigate and provide a way to help those people to avoid or delay the incidence of having CVD(8).

The aim of the present study is to provide a helpful test for the early diagnosis of CVD through measurement of changes in plasma Na⁺, K⁺-ATPase activity to predict CVD long before their development in elderly people.

**SUBJECTS & METHODS**

Patients and normal controls included in this study were selected from King Fahad hospital in Madina area in the Mid-Western region of Saudi Arabia. A total of 115 subjects volunteered, (42 males and 73 females of 50-85 years of age). They were divided into two main groups. The first group consisted of 20 healthy
normal controls. The second group consisted of 95 cardiovascular patients that composed of: 28 patients with valvular heart diseases, (VHD), 22 patients with ischemic heart diseases, (IHD), 15 patient with arrhythmia, (ARRY), 14 patient with heart failure, (HF) and 16 patient with hypertension, (HTN).

After an overnight fast a 4 ml venous blood sample was collected from each subject in lithium heparin, (LH) tubes, and kept at room temperature. Plasma was separated from RBCs. The plasma was used to measure the biochemical parameters using the closed system of DADE BEHRING Dimension (RXL2), [Dimension Clinical System from DADE BEHRING Inc. Newark, DE 19714, USA].

RBC's were washed in isotonic saline three times. Finally, deionized water was added to the sediment and stored at -20°C over night. After thawing, the supernatant was separated by centrifugation at 8000 rpm for 30 min at 4°C, and the ghost, (sediment), was used for estimation of Na\(^+\), K\(^+\)-ATPase activity.

The study included measurements of the following:

- **Determination of sodium, potassium and chloride**\(^{(16)}\).
- **Determination of magnesium**\(^{(17)}\), calcium\(^{(18)}\) and phosphorus\(^{(19)}\).
- **Determination of creatine kinase**\(^{(20)}\), aspartate aminotransferase (AST)\(^{(21)}\), alanine aminotransferase (ALT)\(^{(22)}\), and lactate dehydrogenase (LDH)\(^{(23)}\).

**Statistical analysis**  
Student t-test was used to compare between group means. The p-value was considered to be statistically significant if p≤ 0.05\(^{(24)}\).

**RESULTS**

Table (1) shows plasma Na\(^+\), K\(^+\)-ATPase activity in the studied groups. In the normal control group plasma Na\(^+\), K\(^+\)-ATPase activity was found to be 0.72 \(\pm\) 0.018 \(\cdot\) 10\(^{-3}\) µmol Pi/min.mg. protein. The valvular heart disease group (VHD), has measured 1.80 \(\cdot\) 10\(^{-3}\) \(\pm\) 0.136 \(\cdot\) 10\(^{-3}\) µmol Pi/min.mg. protein, which is significantly higher than the normal control group, (p< 0.001). In the heart failure group (HF), the activity was 1.3 \(\cdot\) 10\(^{-3}\) \(\pm\) 0.117 \(\cdot\) 10\(^{-3}\) µmol Pi/min.mg. protein, which is also significantly higher than the control group, (p< 0.05). The highest activity was found in the ischemic heart disease group (IHD), of value 2.31 \(\cdot\) 10\(^{-3}\) \(\pm\) 0.252 \(\cdot\) 10\(^{-3}\) µmol Pi/min.mg. protein, which is significantly higher than control group, (p< 0.001).

In arrhythmia group (ARRY), the activity was 0.76 \(\cdot\) 10\(^{-3}\) \(\pm\) 0.07 \(\cdot\) 10\(^{-3}\) µmol Pi/min.mg. protein, while in the hypertension group (HTN), it was 0.73 \(\cdot\) 10\(^{-3}\) \(\pm\) 0.14 \(\cdot\) 10\(^{-3}\) µmol Pi/min.mg. protein. Both are non-
significantly different from the normal controls.

Table (1): Plasma Na$^+$, K$^+$-ATPase activity, (mean ± S.D), in the different patients groups as compared to the control group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>Na$^+$, K$^+$-ATPase µmol Pi/min. mg. protein</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>20</td>
<td>0.72 · 10$^{-3}$ ± 0.018 · 10$^{-3}$</td>
<td>----</td>
</tr>
<tr>
<td>VHD</td>
<td>28</td>
<td>1.80 · 10$^{-3}$ ± 0.136 · 10$^{-3}$</td>
<td>p&lt; 0.001</td>
</tr>
<tr>
<td>IHD</td>
<td>22</td>
<td>2.31 · 10$^{-3}$ ± 0.252 · 10$^{-3}$</td>
<td>p&lt; 0.001</td>
</tr>
<tr>
<td>HF</td>
<td>14</td>
<td>1.3 · 10$^{-3}$ ± 0.117 · 10$^{-3}$</td>
<td>p&lt; 0.050</td>
</tr>
<tr>
<td>ARRY</td>
<td>15</td>
<td>0.76 · 10$^{-3}$ ± 0.07 · 10$^{-3}$</td>
<td>N.S.</td>
</tr>
<tr>
<td>HTN</td>
<td>16</td>
<td>0.73 · 10$^{-3}$ ± 0.14 · 10$^{-3}$</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.S. = non-significant

Table (2) shows plasma Na, K, Mg, Ca, Cl, and P in the different patients groups as compared to the normal control group. Non-significant differences have been noticed for plasma sodium and Calcium.

However, plasma potassium of the ischemic heart disease group (IHD), with a value of 3.859 ±0.097 mmol/L was significantly lower than the control group, (p< 0.05). Plasma chloride showed two significantly lower values than the control group: of 65.733± 20.82 mmol/L, (p< 0.05) in the heart failure group (HF), and of 100.645 ± 0.76 mmol/L, (p< 0.05), in the hypertension group (HTN), respectively, while for plasma magnesium only the heart failure group (HF), with value of 0.583 ± 0.159mmol/L, was found highly significant lower than the control group, (p<0.001).

Table (2): Plasma Na, K, Mg, Ca, Cl and P, (mean ± S.D), in the different patients groups as compared to the control group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Na mmol/L</th>
<th>K mmol/L</th>
<th>Mg mmol/L</th>
<th>Ca mmol/L</th>
<th>Cl mmol/L</th>
<th>P mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>138.05±0.71</td>
<td>4.171±0.011</td>
<td>0.793±0.067</td>
<td>2.318±0.039</td>
<td>88.353±8.09</td>
<td>1.147±0.051</td>
</tr>
<tr>
<td>VHD</td>
<td>136.13±0.87</td>
<td>4.056±0.095</td>
<td>0.653±0.064</td>
<td>2.306±0.038</td>
<td>78.624±8.06</td>
<td>1.094±0.034</td>
</tr>
<tr>
<td>IHD</td>
<td>135.59±0.89</td>
<td>3.859*±0.097</td>
<td>0.716±0.076</td>
<td>2.294±0.034</td>
<td>83.791±0.52</td>
<td>1.098±0.068</td>
</tr>
<tr>
<td>HF</td>
<td>135.59±0.86</td>
<td>3.899±0.19</td>
<td>0.583**±0.159</td>
<td>2.230±0.062</td>
<td>65.733±0.82</td>
<td>1.103±0.086</td>
</tr>
<tr>
<td>ARRY</td>
<td>138.08±1.06</td>
<td>4.142±0.13</td>
<td>0.678±0.102</td>
<td>2.249±0.059</td>
<td>81.120±3.56</td>
<td>1.054±0.045</td>
</tr>
<tr>
<td>HTN</td>
<td>137.75±0.96</td>
<td>4.023±0.11</td>
<td>0.819±0.023</td>
<td>2.259±0.031</td>
<td>100.645±0.76</td>
<td>1.036±0.050</td>
</tr>
</tbody>
</table>

* = p< 0.05  ** = p< 0.001
Table (3) shows plasma glucose, urea and creatinine in the different patients groups as compared to the control group. The normal control group showed a plasma glucose value of 5.55 ± 0.195 mmol/L.

Only two groups, namely; the valvular heart disease group (VHD), and the heart failure group (HF), scored significantly higher, (p< 0.05), with values of 7.55 ± 1.03 mmol/L and 9.13 ± 1.25 mmol/L respectively.

With respect to plasma urea the same two groups differed significantly, (p< 0.05), from the normal control group which had a value of 6.857 ± 1.08 mmol/L. The valvular heart disease group (VHD), showed the lowest value of 5.186± 1.933 mmol/L and the heart failure group (HF), showed the highest value of 10.20± 2.25mmol/L.

Only the heart failure group (HF), with plasma creatinine value 162.60 ± 29.18 mmol/L was found to be significantly higher, (p< 0.05), than the normal control value of 105.38 ± 5.18 mmol/L.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Glucose mmol/L</th>
<th>Urea mmol/L</th>
<th>Creatinine mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>5.55 ± 0.195</td>
<td>6.857±1.08</td>
<td>105.38±5.18</td>
</tr>
<tr>
<td>VHD</td>
<td>7.55* ± 1.03</td>
<td>5.186*±1.933</td>
<td>80.96±27.401</td>
</tr>
<tr>
<td>IHD</td>
<td>6.90 ± 0.73</td>
<td>7.23±0.87</td>
<td>111.87±15.73</td>
</tr>
<tr>
<td>HF</td>
<td>9.13 *± 1.25</td>
<td>10.20 *±2.25</td>
<td>162.60*±29.18</td>
</tr>
<tr>
<td>ARRY</td>
<td>6.23 ± 1.10</td>
<td>7.93±1.43</td>
<td>100.55±23.90</td>
</tr>
<tr>
<td>HTN</td>
<td>6.18 ± 0.45</td>
<td>6.73±1.24</td>
<td>90.75±13.14</td>
</tr>
</tbody>
</table>

* = p< 0.05
** = p< 0.001

Table (4) shows the plasma lipid profile of the different patients groups as compared to the normal control group. Plasma triglycerides of 1.67 ± 0.14 mmol/L was found in the normal control group. Only the ischemic heart disease group (IHD), with the lower value of 1.11 ± 0.11 mmol/L, was found to be significantly different, (p< 0.05).

Plasma cholesterol was found to be 4.99 ± 0.315 mmol/L in the normal control group. Only the heart failure group (HF), with a value of 5.62 ± 0.44 mmol/L, was found to be significantly higher than the control group, (p< 0.05).

Plasma HDL value of 0.92 ± 0.14 mmol/L was found in the normal control group. Two of the patient group had significantly higher values namely; the hypertension group (HTN), with 1.37 ± 0.23 mmol/L, (p< 0.05), and the heart failure group (HF), with 1.57 ± 0.57 mmol/L, (p< 0.01). As for plasma LDL a value of 3.163 ± 1.52 mmol/L was recorded for the normal control group, with a significantly higher value of 3.367± 1.023 mmol/L for the arrhythmia group (Arry), (p< 0.050).
Table (4): Plasma Lipid Profile, (mean ± S.D), in the different patients groups as compared to the control group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Triglycerides mmol/L</th>
<th>Cholesterol mmol/L</th>
<th>HDL mmol/L</th>
<th>LDL mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>1.67± 0.14</td>
<td>4.99 ± 0.315</td>
<td>0.92 ± 0.14</td>
<td>3.163 ± 1.52</td>
</tr>
<tr>
<td>VHD</td>
<td>1.45± 0.18</td>
<td>4.55± 0.18</td>
<td>1.07± 0.10</td>
<td>2.853± 0.796</td>
</tr>
<tr>
<td>IHD</td>
<td>1.11 *± 0.11</td>
<td>4.29 ± 0.31</td>
<td>1.24 ± 0.36</td>
<td>2.731 ± 1.142</td>
</tr>
<tr>
<td>HF</td>
<td>1.34± 0.18</td>
<td>5.62*± 0.44</td>
<td>1.57**± 0.57</td>
<td>3.140± 1.412</td>
</tr>
<tr>
<td>ARRY</td>
<td>1.38± 0.12</td>
<td>4.60± 0.35</td>
<td>0.91± 0.11</td>
<td>3.367*± 1.023</td>
</tr>
<tr>
<td>HTN</td>
<td>1.51± 0.19</td>
<td>4.92± 0.19</td>
<td>1.37*± 0.23</td>
<td>2.750± 1.007</td>
</tr>
</tbody>
</table>

* = p< 0.05  
** = p< 0.001

Table (5): Plasma CK, LDH, AST and ALT, (mean ± S.D), in the different patients groups as compared to the control group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CK U/L</th>
<th>LDH U/L</th>
<th>AST U/L</th>
<th>ALT U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>82.62±10.50</td>
<td>184.195±7.44</td>
<td>24.52±1.34</td>
<td>37.69±3.42</td>
</tr>
<tr>
<td>VHD</td>
<td>105.68±37.69</td>
<td>227.35±14.57</td>
<td>29.82±2.84</td>
<td>38.217±2.73</td>
</tr>
<tr>
<td>IHD</td>
<td>56.818±6.61</td>
<td>190.05±9.36</td>
<td>24.00±1.82</td>
<td>35.217±4.38</td>
</tr>
<tr>
<td>HF</td>
<td>433.714*** ± 175.49</td>
<td>263.714*** ± 42.94</td>
<td>49.86*** ± 14.48</td>
<td>46.657*** ± 7.96</td>
</tr>
<tr>
<td>ARRY</td>
<td>57.27±12.29</td>
<td>204.046±18.73</td>
<td>40.82*** ± 7.87</td>
<td>53.591*** ± 11.14</td>
</tr>
<tr>
<td>HTN</td>
<td>44.688±6.18</td>
<td>172.844±10.31</td>
<td>22.94±2.12</td>
<td>33.675±3.79</td>
</tr>
</tbody>
</table>

* = p< 0.05  
** = p< 0.005  
*** = p< 0.001
DISCUSSION

Cardiovascular disease (CVD), is common in the elderly and affect 50% of those over the age of 65 years. It is the leading cause of death for the age range 65-74 years, (Lye et al., 2000). Hypercholesterolemia has been recognized as direct and independent risk factors for CVD and recognized as the most important modifiable risk factor where early diagnosis and therapy can reduce incidence of CVD events. 

Saudi Arabia has witnessed significant urbanization over the last 40 years with significant improvement in social and economic status resulting in huge changes in lifestyle, accommodating more of the "Western" style with respect to diet and physical activity. Saudi Arabia's ecology results in wide variations in the life style and food consumption patterns which might be a major underlying cause of the variation and high prevalence of coronary artery disease risk factors(26). Another survey showed that features of insulin resistance syndrome (IRS), are widely prevalent among the Saudi population over the age of 40 years. IRS is a probable significant contributor to the pathologic process of CVD among the Saudi population, especially in view of the low prevalence of hypercholesterolemia(27).

Several studies have shown that Na⁺, K⁺-ATPase activity decrease in heart tissues in CVD patients(28 & 29). In the present study Na⁺, K⁺-ATPase activity was found to be significantly higher in 3 out of 5 CVD patients groups. This contradicts the results of Dai et al(30), who found that Na⁺, K⁺-ATPase activity was significantly lower by 16%, in CVD. The increase in Na⁺, K⁺-ATPase activity of most CVD patients groups could be attributed to the pharmacological and surgical treatment, since almost 95% of the patients were already treated surgically or pharmaceutically, which agrees well with Vrbjar et al., and Fuller et al.(31 & 32). In arrhythmia (ARRY), and hypertensive (HTN), patients Na⁺, K⁺-ATPase activity showed almost the same activity of age matched healthy normal controls, which agrees well with results from Zavecz & Dutta, and Dai et al.(33 & 30).

All patient groups varied in showing significant decrease in various plasma electrolytes. This agrees with the fact that plasma electrolytes remain constant or slightly decreases with advancing age(34).

Plasma glucose was found to be significantly higher in heart the failure (HF), group than the normal control group. This agrees well with the findings of Iribarren et al.(35), who stated that poor glycemic control in elderly population may be associated with an increased risk of heart failure. As for the significant increase in plasma glucose level in the valvular heart disease (VHD), it goes well with the study of Podlesny(36) who found that diabetes has an influence on the morphology of the heart seen as hypertrophy of the left ventricle.

Plasma urea and creatinine were found highest in the heart failure group. This goes well with the study of Nilsson et al.(37), who showed that heart failure is associated with
impaired creatinine clearance and increased urea and creatinine.

The lowest triglyceride found in ischemic heart disease group could be attributed to treatment, since its increase is one of the factors that induce ischemic heart diseases\(^{(38)}\).

The highly significant increase in plasma cholesterol found in the heart failure group agrees with Nilsson et al.\(^{(37)}\) who showed that cholesterol increases in congestive heart failure.

Low HDL is an important risk factor for CVD death in the elderly; high HDL has significant protective effect against coronary artery disease\(^{(39)}\). The highly significant increase in plasma HDL in the heart failure and the hypertension groups points to its control by treatment. However, plasma LDL showed a non-significant decreased except in the arrhythmia group which showed a significantly higher value pointing again to the variable effects of treatment.

Cardiac enzyme marker CK, LDH, AST and ALT showed significant higher activities in heart failure patients. ALT showed an unexplained sole significant increase in arrhythmia patients.

In view of the results of all the classical laboratory diagnostic tests it could be conclude that plasma Na\(^+\), K\(^+\)-ATPase activity has been proved as a helpful additional laboratory diagnostic test for the early diagnosis and prognosis of cardiovascular diseases while advancing in age.

REFERENCES


ملخص باللغة العربية

تعتبر أمراض القلب الوعائية السبب الأساسي للموت المبكر بما نسبته حوالي 25% من الوفيات على مستوى العالم.

وأمراض القلب الوعائية هي النتيجة النهائية لكامل عدد من عوامل الخطورة كالتقدم في السن والسمة وأرتفاع نسبة الكوليسترول في الدم وأرتفاع ضغط الدم ومرض السكري والتدخين ونسبة الحياة.

وهذا العامل الأخير قد شهد تحسناً هائلاً بالمملكة العربية السعودية خلال الأربعين سنة الماضية نتيجة التقدم الباهت وأنه تجاه نهج الحياة الغربية مما أدى إلى زيادة كافة أنواع المخاطر المذكورة.

وهدف هذه الدراسة هو تقديم اختبار يمكن أن يساعد على التشخيص المبكر لأمراض القلب الوعائية في بازارنا الدم في الأنواع المختلفة من أمراض Na⁺، K⁺-ATPase وذلك من خلال دراسة نشاط خميرة القلب الوعائية مثل حالات فشل القلب وأحتقان القلب وخلال قصص القلب وغيرها ومحاولة ربط النتائج بالقياسات المعملية الأخرى مثل قياسات خمائر وتواتر القلب ووظائف الدم ووظائف الكلى وفترة الطلق.

أشارت هذه الدراسة على 95 مريضاً بأمراض مختلفة من أمراض القلب الوعائية بالإضافة إلى 20 من الأصحاء وتراوحت أعمارهم من 50 و 65 سنة من الجنسين.

وتتنوع النتائج إلى زيادة مرجعية في نشاط خميرة Na⁺، K⁺-ATPase في غالبية المرضى بينما أشارت غالبية القياسات الأخرى إلى النمط المعقول في مثل هذه الحالات.

ويمكن القول بوجود أخطاء اختبار خميرة Na⁺، K⁺-ATPase في بازارنا الدم كاختبار للكشف المبكر عن أمراض القلب الوعائية.