

# Assessment of Anti-streptokinase Antibody in Patients with Heart Diseases and Normal Subjects

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## SUMMARY

**Background:** Streptokinase, which is injected intravenously with a standard dose of 1.5 MIU, is the most widely used thrombolytic agent around the world. What is so important about this bioproduct is the level of anti-streptokinase (anti-sk) antibody in the population, which is directly correlated to the incidence of streptococcal infections in that population. **Objective:** Since Iran is an endemic area for streptococcal infections, this study was conducted to assess the anti-sk level in an Iranian population. **Materials and Methods:** 97 males and 47 females referred to Modarress Hospital of Tehran for coronary angiography and cardiac catheterization were included. 10 ml of venous blood was taken before angiographies from each patient. According to the angiography reports, the patients were divided into three groups: Coronary Artery Diseases (CAD, n=95), Rheumatic Heart Disease (RHD, n=19) and normal coronaries (n=30). The anti-sk antibody level was assessed in the serum samples of all patients using Enzyme Linked Immunosorbant Assay. **Results:** In 23.2% of patients with CAD, 40% of normal coronaries and 73.7% of patients with RHD, the serum samples contained more than 2 arbitrary units (AU) of anti-SK antibody which regarded as high levels. There was no significant difference between the anti-sk level of patients with CAD and normal coronaries ( $2.03 \pm 3.02$  AUs vs.  $2.52 \pm 2.23$  AU), but the level of antibody in RHD group ( $8.16 \pm 10.1$  AU) was significantly higher than other groups ( $p < 0.05$ ). No significant correlation was observed between antibody levels and the age or gender of patients. **Conclusion:** We concluded that the level of anti-sk antibody is high in Iranian population as compared to other endemic areas for

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streptococcal infections. Also we found no relation between the level of antibody and sex and age of patients. This study accentuated the necessity of assessment of drug efficacy in endemic areas for streptococcal infections especially in those patients with valvular heart disease.

**Keywords: Antibody, Heart Disease, ELISA, Streptokinase**

## INTRODUCTION

Thrombolysis by using thrombolytic drugs is accepted as a standard treatment in acute myocardial infarction (AMI), since it is believed that the main cause of AMI in more than 85% of involved cases is thrombosis formation in coronary arteries (1-5). It is proved that using thrombolytic agents can reduce mortality rates approximately by 30% and improve left ventricular function, restrict myocardial necrosis and improve patient outcome (6-9). The most commonly used thrombolytic agents are recombinant tissue plasminogen activator (rtPA) and streptokinase (SK). SK is always the first choice since it costs less than others unless a contraindication is proved (10,11).

SK therapy is not always successful. This may, among other causes, be due to the presence of IgG anti-sk antibodies in the patients (12). These antibodies may be present as a result of previous infection with streptococci or previous treatment with SK (13-16). Different studies in populations of endemic areas for streptococcal infections (17-19) show that the level of anti-sk antibodies is higher compared to those populations with low incidence of infections (20-22). It is proposed that the higher level of antibodies may be associated with lower clinical efficacy and increased risk of hypersensitivity reactions (23-26) although there are some controversies (27-30).

As Iran is regarded as an endemic area for streptococcal infections, and because of the importance of anti-sk antibodies in relation to drug efficacy, this study was conducted to analyze the level of anti-sk antibodies in a group of patients with heart diseases referred to Modarress Hospital, Tehran, Iran, for angiographies.

## SUBJECTS AND METHODS

In this cross-sectional study 170 patients were included at first. The inclusion criteria included: Never prescribed with streptokinase, Not used to immunosuppressive or immunostimulatory drugs for a long time in the past. Before angiography 10ml of venous blood was taken from all the patients and sera were isolated. Using anti-streptolysin O (ASO) and

rheumatoid factor (RF) laboratory tests, all the patients with rheumatoid factor or those with ASO > 200 were excluded. Finally 144 patients including 47 females and 97 males remained to be studied. The patients were divided into three main groups based on angiography results including: 95 patients with coronary artery disease (CAD), mean age  $57.89 \pm 9.46$  yrs., 19 patients with rheumatic heart disease (RHD), mean age  $43.22 \pm 10.85$  yrs., 30 normal coronaries, mean age  $50.66 \pm 9.44$  yrs. An indirect ELISA assay was designed for detection of IgG anti-sk antibody level in the serum samples. The procedure was briefly as follows: The microtiter plate was coated with 100 $\mu$ l of heberkinasa at a concentration of 6 $\mu$ g/ml. After an overnight incubation at 4 $^{\circ}$ c and washing 3x with phosphate buffered saline (PBS), 100 $\mu$ l of serum samples were added in 4 serial dilutions starting with 1/400. Also serial dilutions of a pooled serum collected from several patients who had received streptokinase before, were included in the plate. The plate was then incubated for an hour at 37 $^{\circ}$ c and then washed 3x with PBS. In the next step, 100 $\mu$ l of anti-human IgG conjugated to HRP diluted at 1/28000 was added to the wells. After incubation for an hour at 25 $^{\circ}$ c and washing 3x with PBS, substrate solution (8mg OPD per 12 ml of double distilled water +5 $\mu$ l hydrogen peroxide 30%) was added and the plate was incubated for 10 min at room temperature in dark. The reaction was stopped with 100 $\mu$ l of 0.5M H<sub>2</sub>SO<sub>4</sub> and the result was analyzed with an ELISA reader at 490nm (MR7000-Dynatech) to get the OD values of every individual serum at different dilutions. The titration curve of the pooled positive serum was used as an intralaboratory standard. The OD value of a specified dilution of this serum (1/102400) was assigned 1 arbitrary unit (AU) and the level of anti-sk antibody in all the sera at 1/400 dilution, was quantified by using arbitrary units. The antibody level less than 2 AU was considered as low, because all the sera with less than 2 AU should be diluted less than 800 times to reach the lowest antibody level. The antibody level of 2AU or more was considered as high, because all the sera with 2AU or more antibodies should be diluted more than 800 times to reach the lowest antibody level.

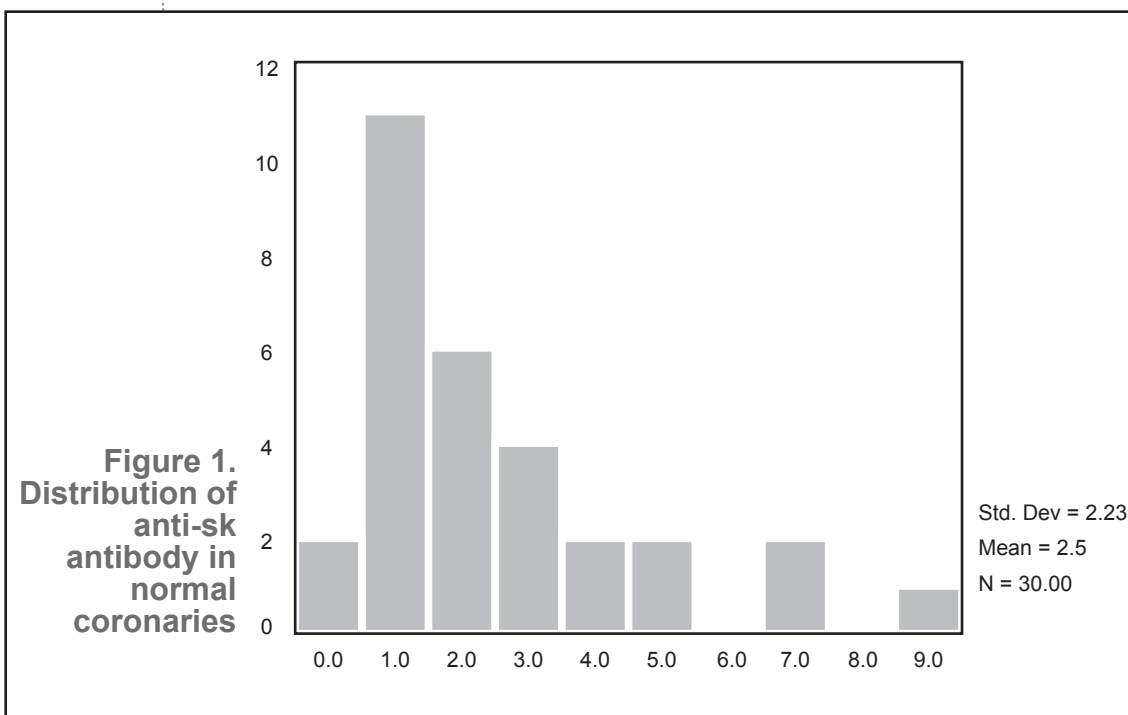
The designed system was accurately reproducible as the intraassay coefficient of variation (CV) and interassay CV were less than 10%.

Data analysis was performed using nonparametric analytical tests: Kruskal-Wallis and Mann-Whitney U. The correlation was analyzed using Spearman's correlation test.

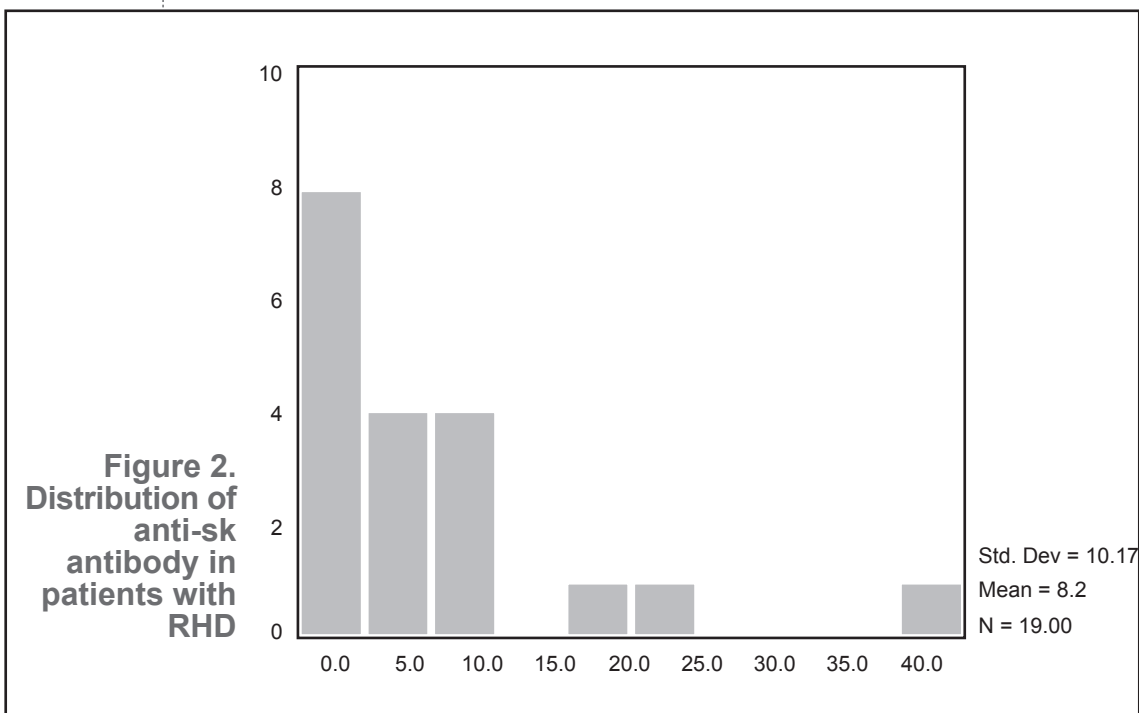
## RESULTS

The comparison of anti-SK antibody in patients with ASO>200 (mean= 4.93 AU) and those with ASO < 200 (mean= 2.94 AU), showed that there

is a significant difference between these two groups ( $P= 0.002$ ). As shown in the figures 1-3, the distribution of anti-sk antibody in the population studied, is not normal and is skewed towards higher levels.

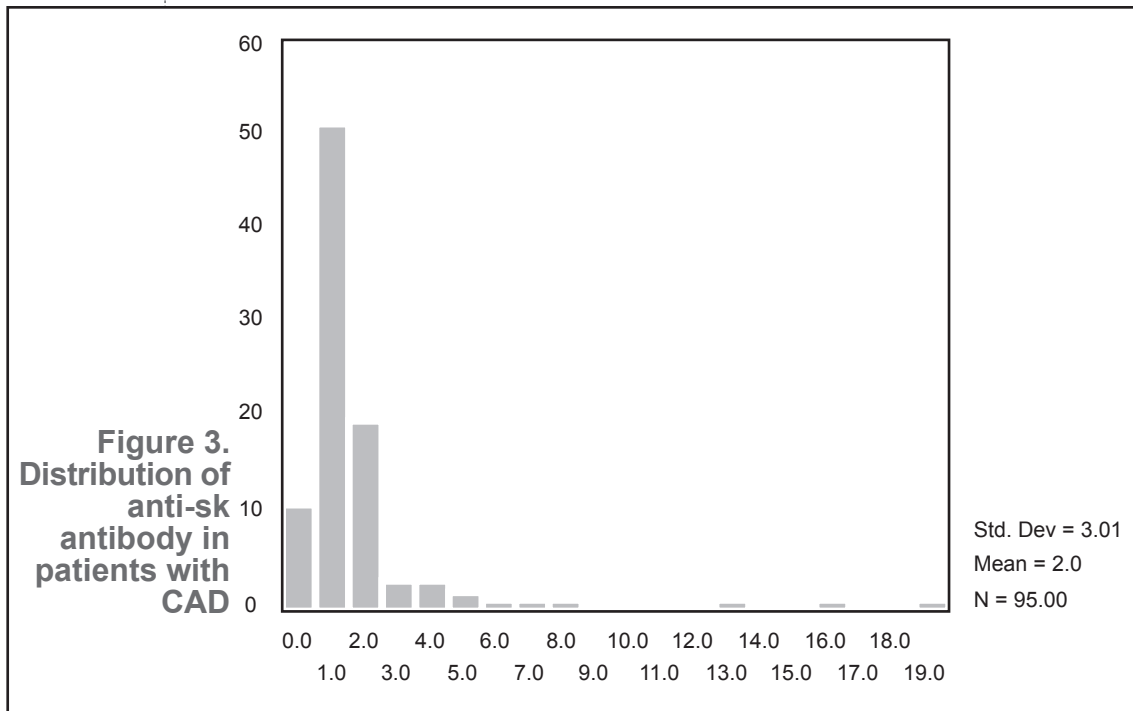


The level of anti-sk antibody of patients with RHD (mean=8.165 AU) is significantly higher (approximately 4 times) than those with CAD (mean=2.032 AU,  $P \approx 0.000$ ) and normal coronaries (mean=2.527 AU,  $P=$



0.009), but there is no significant difference between the anti-sk level of patients with CAD and normal coronaries.

Frequency studies showed that in 73.8% of patients with RHD, 40% of



normal coronaries and 23.2% of patients with CAD the anti-sk antibody level is 2 arbitrary units or higher. Also the level of anti-sk antibody of 3 patients with CAD was more than 13 AUs.

The level of anti-sk antibody had no significant correlation with the age of patients as analyzed by Spearman's correlation coefficient in all the three main groups:

In RHD patients:  $r = 0.24$ ,  $P = 0.34$ , in CAD patients:  $r = -0.06$ ,  $P = 0.57$  and in normal coronaries:  $r = 0.02$ ,  $P = 0.94$

The difference between the level of anti-sk antibody in females (mean= 4.22 AU) and males (mean= 2.38 AU) was not significant.

## DISCUSSION

In this study we showed that there is a significant difference between the anti-sk antibody level of patients with  $ASO > 200$  (as a sign of recent streptococcal infection) and those with  $ASO < 200$ . This confirmed the results of Buchalter and Juhlin (13,24). So we excluded the patients with  $ASO > 200$  in order to assess the anti-sk antibody level with more accuracy.

Our results showed that the distribution of anti-sk level in the population is not in the normal range and is skewed towards higher levels, as studied

in other populations (20,22,27,31), so as the anti-sk antibody level in 23.2% of patients with CAD, 40% of normal coronaries and 73.7% of patients with RHD, is 2 AUs or higher. These results are not comparable to previous reports because the units used to quantify the level of antibody are arbitrary instead of international units, but propose the high level of anti-sk antibody in Iranian population as a result of high streptococcal infection incidence. It should be mentioned that there is a controversy about the effect of higher levels of antibody on drug efficacy. As proposed by Shaila and Fears, the levels of anti-sk antibody before therapy has no effect on the reperfusion rate (18, 28), but Juhlin and Gemmill have shown the reverse results (24, 27). Some of the reasons proposed to interpret these discrepancies are as follows:

- The anti-SK antibodies present in the blood have no effect on the function of the drug (18),
- After neutralization, the level of drug left in the blood is sufficient to fulfill its function perfectly. This means that in spite of the high level of antibody, the standard dose of sk is high enough to fulfill its function (28),
- Different techniques used to probe the reperfusion rate after therapy, including angiography and vectorcardiography, has caused different conclusions. Juhlin believes that vectorcardiography is more efficient than angiography in recording the reperfusion rate. Therefore, his results that: "the higher level of anti-sk antibody has an adverse effect on drug efficacy" is more reliable in his view (24). In this regard, it is necessary to assess the drug efficacy in Iranian population.

Also in this study we showed that the mean level of anti-sk antibody in patients with RHD is significantly higher than other groups. Frequency studies showed that the anti-sk level in 73.7% of these patients is higher than 2 arbitrary units. Our results are newly proposed but are not unexpected because of the known role of streptococcal infections in the etiology of this disease.

There was no significant difference between the level of anti-sk antibody of patients with CAD and those with normal coronaries. This is the same as other studies (13,20,26). The only difference is that in our study the comparison is made between the patients with coronary artery disease without acute myocardial infarction and those suspected to have CAD but proved to be normal after angiography. In other studies, the comparison is made between patients with AMI and normal volunteers.

Also there was no significant difference between the mean level of anti-sk antibody of males and females and there is no correlation between the anti-sk level and the age of patients as showed in other studies (20, 28). This eliminates any assumption that the level of antibody may be lower in older patients and so the drug efficacy is not affected in those. This is also true

for the sex of patients.

In conclusion, we propose that the level of anti-SK antibody is high in Iranian population. This may be due to the high incidence of streptococcal infections in our country. This is consistent with other studies in endemic areas for streptococcal infections. Therefore, it is necessary to assess the drug efficacy in Iranian population. Also we showed the high level of anti-sk antibody in patients with RHD, which proposes the lower drug efficacy used with standard dosage scheme in this group compared to other ones. So it is necessary to assess the drug efficacy in this group, too.

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