Do high levels of antistreptokinase limit the efficacy of streptokinase in patients with myocardial infarction?

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Abstract

BACKGROUND: Antistreptokinase antibody in serum of people who had been exposed to streptococcal infections may interfere with thrombolytic effects of streptokinase. Streptokinase is the only thrombolytic medication in Iran, and is the first line treatment in myocardial infection. Considering the high prevalence of streptococcal infections in Iran as compared to developed countries, the high levels of serum antibody might neutralize streptokinase.

METHODS: Serum levels of antistreptokinase antibody of 126 people with myocardial infarction who went to Noor Hospital (Isfahan, Iran) were measured before administrating streptokinase. The effects of the drug were then evaluated and compared by considering the consequent echocardiographic (ECG) changes during hospitalization.

RESULTS: In 17 out of 126 patients (13.5%), the antibody levels were high and the drug did not have any effects. This number is 2.5 times more than the values in references. In 25 patients, among whom 3 had high levels of antistreptokinase antibody, the drug was effective.

CONCLUSION: Considering the lack of relationship between high levels of antistreptokinase antibody and the efficacy of streptokinase in patients with myocardial infarction in this study, studies with larger sample size and more objective criteria, such as serum fibrinogen as the indicator of streptokinase efficacy, are recommended.

Keywords: Coronary Artery Diseases, Thrombolytic Treatment, Streptokinase, Antistreptokinase Antibody.

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Introduction

Myocardial infarction is one of the most prevalent and deadliest diseases which occurs following a sudden decrease in coronary artery flow after thrombotic occlusion of already atherosclerotic arteries.[1] Nearly 25% of deaths in Americans over 35 years old is caused by myocardial infarction, and about half of such deaths are sudden and occur before reaching hospital.[2,3] Over 90% of myocardial infarction cases occur in total occlusion of coronary arteries, and only less than 5% occur in normal coronary arteries. In case of lack of appropriate and timely perfusion, the risk of heart failure following myocardial infarction will be increased by 2-6 times.[4-7]

During ischemia and myocardial infarction, two mechanisms increase the demand and reduce the supply. Thrombosis plays an important role in causing infarction because it has a role in reducing supply in 90% of cases.[3,4] One of the therapeutic procedures in infarction is reperfusion, which can happen in 3 different ways including coronary artery bypass, percutaneous transluminal coronary angioplasty (PTCA), and thrombolytic therapy.[8] Streptokinase, as the only thrombolytic medicine in Iran, is a protein produced by different species of hemolytic streptococcus.[9,10] Most people have streptokinase neutralizing antibodies in their serum which can be caused by previous infection with β-hemolytic streptococcus.

The prevalence of streptococcal infections is high in Iran just like other developing countries.[11] Therefore, during thrombolytic therapy, sufficient streptokinase should be infused in order to neutralize these antibodies. In some cases however, i.e. about 5% of patients with myocardial infarction, the increased dose is not effective either.[12]

The present study aimed to evaluate the efficacy of streptokinase in patients with myocardial infarction in a society with a high serum level of antistreptokinase antibodies.

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Materials and Methods

Regarding to 5% incidenc of high level of antistreptokinase antibody in patients with acute MI a total number of 126 patients with myocardial infarction at Noor Hospital (Isfahan University of Medical Sciences, Isfahan, Iran) were selected. All patients went to the hospital within the first 6 hours of having chest pain, so Q wave was not present in their first echocardiography (ECG). Cardiac enzymes, such as lactate dehydrogenase (LDH) and creatine phosphokinase (CPK), were elevated in all patients during hospitalization.

Before administration of streptokinase, a 5-ml venous blood sample was taken from each patient and centrifuged at 1500 revolutions per minute (rpm) for 10 minutes to separate serum. The samples were then stored at -70°C. Enzyme-linked immunosorbent assay (ELISA) was used to study serum levels of antistreptokinase (there are no kits for this purpose).[13,14]

In order to perform ELISA tests, 96-well microplates (Nunc Microplate 96 wells Polysorp) were used. The infused streptokinase vials (Kabi Pharmacia Inc.) were used as antigen. Then, 100 µl of the prepared suspension (100 U/ml) were added to each well in the microplates, and they were incubated at 4°C for 24 hours. Afterwards, the excess solution was removed from the wells, and 250 µl of phosphate buffered saline (PBS) containing 1% of bovine serum albumin (BSA) (PBS-1% BSA) were added to prevent nonspecific binding in later stages. After 30-minute incubation at 37°C, plates were washed with PBS 0.1% Tween-20 four times. Then, 100 µl of 1:40 diluted serum of patients or healthy people was added to each well. The plates were subsequently washed 3 times. Finally, 100 µl of rabbit antihuman immunoglobulin G (IgG) conjugated with horseradish peroxidase (Daco Company, Denmark) at dilution of 1:6000 were added.

The plates were incubated at 37°C for an hour, washed for 3 times, and finally 100 µl of peroxidase substrate was added to each well. The solution contained ortho phenylenediamine dichloride (OPD), which was made by dissolving a 30-mg tablet in 10 µl of citrated PBS at pH 5, and mixed with 2 µl of hydrogen peroxide.

After 10 minutes of incubation at 37°C, 50 µl of H_2SO_4 (0.5 M) were added to each well to stop the reaction. The amount of optical density (OD) was read at a wavelength of 492 nm using an ELISA reader (Bio-Tek Instruments Inc., Winooski, VT, USA).[14-19]

In several wells, samples of positive and negative control serum were used. For negative control, serum of people with no history of streptococcal infection and antistreptolysin O (ASO) titer of less than 100 Todd units was used. For positive control, serum of people who had had a streptococcal infection based on their physician's confirmation and paraclinical reports more than 15 days before, and had a high titer of ASO (> 500 Todd units) was used. In some cases, monoclonal antistreptokinase antibody was used as positive control. There was no difference between the two groups (high ASO and monoclonal antibody) of positive control. Finally, using the following formula:

$$\text{Cut off} = \frac{\textit{minimum optical density in positive serum sample}}{\textit{maximum optical density in negative serum sample}}$$

the range of serum antibody was obtained. Cut-off is a scientific method of ELISA to measure antibody titer in serum. Using serum levels of antistreptokinase, absorption curve, and optical density, samples with titers more than the cut-off point were considered positive. Furthermore, serial ECGs were taken during the hospitalization to monitor the appearance of Q wave (which is a sign of efficacy or lack of efficacy of streptokinase). The results were analyzed using Fisher's exact test. In this study, 18 healthy people with no myocardial infarction history were studied as control group.

Results

Out of 126 serum samples taken from patients with myocardial infarction prior to administration of streptokinase, 20 people (16%) had high titer of antistreptokinase antibody. In 25 people (20%), Q wave did not appear in serial ECGs for 3 days. Moreover, changes in ECG did not support myocardial infarction which showed the efficacy of the therapy.

In total, medication was not effective in 17 people (13.5%). Based on statistical tests, there was no significant relationship between high serum antibody and efficacy of streptokinase (P > 0.05) (Table 1).

Table 1. Frequency of efficacy of streptokinase on antistreptokinase antibody serum level in patients with myocardial infarction

	Efficacy of streptokinase	
Antibody titer	**Effective**	**Ineffective**
Serum positive	3	17
Serum negative	22	74

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Discussion

Thrombolytic therapy is used extensively for perfusion. In Iran, the only medication available is streptokinase. That is why studying the role and efficacy of streptokinase is important. Several studies have evaluated the efficiency of streptokinase and factors preventing its effects. However, they reported contradictory results. Some studies suggested that streptokinase therapy should be applied at least 3 to 6 months after observing high levels of antistreptokinase antibody due to a recent streptococcal pharyngitis. Receiving streptokinase 2 days to 2 years or even 4 years later would cause resistance and reduced efficacy of streptokinase. In addition, if re-administration of streptokinase before 6 months is not permitted.[7]

Several studies have shown that antistreptokinase antibody does not prevent the efficacy of streptokinase on reperfusion. Feares et al. used micro-radioimmunoassay to measure serum levels of antistreptokinase IgG in qualified patients who had received streptokinase. The efficacy of the drum was assessed by angiography at 1 and 24 hours after the administration of streptokinase. The results showed a wide range of resistance to streptokinase in patients while none of them had used the drug. Most patients had a little resistance to streptokinase, and its efficacy was not significantly reduced by antistreptokinase antibody IgG. However, the resistance remained for 4 years.[15]

A previous study used dilution neutralization assay and ELISA to measure antistreptokinase. It also employed in vitro fibrin plate lysis assay to assess streptokinase efficacy. The results showed that the prevalence of ant streptococcal antibody in general population and in patients who referred with first-time myocardial infarction was not clear. None of the patients had a high level of ant streptokinase antibody with in vitro fibrinolytic resistance to streptokinase, and most of them responded to the standard dose of streptokinase despite previous streptococcal infections.[16] In this study, resistance to streptokinase was seen during the 2-4 days after treatment to 2 or even 4 years after treatment.[16]

A study in Utah concluded that the presence of ant streptokinase did not have an important effect on the response to treatment with streptokinase.[15] Therefore, even in extensive studies, there have no definite results, which can be attributed to different levels of antistreptokinase antibody in different studies.

In the present study, no ST segment resolution atleast 70% compare to baseline of ECG after 90 minute of SK infusion and the appearance of Q wave after administration of streptokinase in patients with myocardial infarction was considered as failure to prevent infarction. Q wave appeared in 80% (n = 101) of patients despite receiving streptokinase. This is while some patients, despite having symptoms of myocardial infarction (elevated ST segment in ECG for 2 mm in anterior precordial leads or 1 mm in standard leads along with chest pain for more than 30 minutes), Q wave did not appear despite no administering streptokinase, and patients did not experience myocardial infarction, which can be attributed to removing spasm or automatic lysis due to activation of anticoagulation system.[19]

It is noteworthy that the importance of streptokinase is not just for the reperfusion, but for prevention of remodeling, arrhythmias, and heart failure.[1] In this study, while Q wave appeared more in patients with higher levels of antistreptokinase antibody (17 out of 20 people), it was even seen in patients with lower levels of antibody (84 out of 106). Therefore, the relationship between antibody level and streptokinase effect is doubted. Q wave might have appeared in patients who received streptokinase at the right time and had a low level of antistreptokinase antibody due to several reasons. Other thrombolytic agents like plasminogen activator inhibitor-1 (tPAI1) or lipoproteins which are molecularly similar to plasminogen, and prevent the formation of plasmin should be considered.[4,18,19] Fibrinogen levels must be studied to see whether thrombolytic therapy can significantly reduce fibrinogen levels or not.

The number of patients was determined based on the 5% incidence of high level of antistreptokinase antibody while in this study, the high level of antistreptokinase antibody was about 13.5%. Therefore, it is recommended to conduct another study with a larger sample size.

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Conflict of Interests

Authors have no conflict of interests.

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