Conclusions

1. The diabetic retinopathy development is linked to a prolonged VEP latency period (P100), lowering the N75-P100 amplitude, as well as to shortening the recovery period of retina's functional capacity after photo stress.

2. The cerebellar trans-cranial magnetic stimulation facilitates a faster recovery of the retina's functional capacity in response to photo stress in diabetic patients with retinopathy.

3. The periodical cerebellar trans-cranial stimulations may be considered as a prevention method as well as a method of treatment of diabetic retinopathy.

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Production of anti-prostate specific antigen coated tubes

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Abstract

Background: Immunoassay is an appropriate method for measuring hormones and other protein compounds like a prostate-specific antigen (PSA). This antigen is a tumor marker of prostate cancer which can be identified by screening. Among the numerous available solid phases a coated tube is the most popular one.

Material and methods: We have used a monoclonal antibody (mAb) against the PSA. One of the pairs of monoclonal antibodies has been used for preparing the PSA tracer by labeling to radioactive iodine (^{125}I) and the other one – for the coating of polystyrene tubes. In the solid phase method we have used the adsorption technique for coating the monoclonal antibody in polystyrene tubes. We have used adsorption as a mode of coating and plastic tubes as a solid phase. The reaction takes place between the antigen molecule in the patient serum and the two antibodies (anti-PSA mAb) in the tracer and coated tubes, and hence these assays are called solid phase assays. The determination of PSA is based on the immunoradiometric assay (IRMA). The samples or standards are incubated in monoclonal antibody-coated tubes with the second ^{125}I-labelled antibody. After the incubation period (two hours) the liquid contents of the tubes are aspirated and washed.

Results: After running the assay the results have showed a correlation between the concentrations of the serum PSA tested by the locally made coated tubes and the commercial ones. So, we can use the locally made reagents to measure the PSA not only for the laboratories performing a large number of tests, but also for the whole country in screening the prostate cancer which will surely be a cost-effective project. Finally, as the method is isotopic, it is more robust compared to the non-isotopic markers and presents a true immunoassay.

Conclusions: Many solid phases have specific reactive groups which can be activated by a variety of biochemical methods. This research shows that we can manufacture the cost-effective IRMA kits by producing locally the antibody-coated tubes for PSA. And they can be used in mass screening of the prostate cancer for men over 50 in any country. Finally the same protocol for coating monoclonal antibodies can be used for other tumor markers like CEA, CA15-3 and AFP if we have their specific monoclonal antibodies.

Key words: solid phase, coated tubes, immunoassay, prostate-specific antigen.

Introduction

The first solid phase of radioimmunoassay was reported by K.Catt and G.Treagear in the Science Journal at the earlier stage of prostate specific antigen (PSA) development [2]. An important aspect of the immune response is the production of antibodies or immunoglobulins by B-cells (B-lymphocytes). Serum, when it contains specific antibodies, is referred to as antiserum. All the antibodies have the same basic structure of 4 polypeptide chains: two identical "light" chains and two identical "heavy" chains linked together by disulfide bonds in a distinctive Y conformation. Immunoglobulins are divided into classes (IgG, IgM, IgE, IgA and IgD) and subclasses (IgG1,

IgG2, etc). IgG class immunoglobulins constitute about 75% of total serum immunoglobulins. The antibodies that are produced by hyper-immunization are predominantly IgG, occasionally with IgM. And IgM antibodies are often less stable [2, 8].

The initial stimulation of antibody production is usually achieved in an animal after the injection of immunogen. The administration of immunogen stimulates different cells of the immune system, giving rise to a mixed population of antibodies derived from a number of B-lymphocyte clones (polyclonal). The serum taken from an immunized animal containing these antibodies is referred to as a polyclonal antiserum [3, 5].

In the recent years, the usage of monoclonal antibody (mcAb) in the immunoassay has rapidly been established along with involving a variety of approaches. Kohler and Milstein's original method was to fuse B-cells antibodies with neoplastic tumor cells (B-cell myeloma), producing immortal hybrid cells.

There are three factors necessary for judicious selection of antibodies:

1. Concentration of binding sites
2. Affinity
3. Specificity

It is, therefore, important to detail or indicate the aspects of the above in order to characterize all antibodies if they are to be used as immune reagents. The most practical tests are:

1. Determination of titer. This is a measure of both concentration and affinity.

2. Scatchard's analysis of binding curve. This gives the details of both affinity and number of binding sites.

3. Cross-reaction studies with structurally related molecules. This can give the the information to describe the type and degree of specificity.

In this research we have used the pairs of monoclonal Ab from a commercial company. One of them has been used before for making the tracer with ^{125}I and the other one has been used for coating on a solid phase. In solid phase assays, the antibody is physically adsorbed on or chemically coupled to a solid phase. The reaction takes place between the antigen molecules on the liquid phase and the antibody on the solid phase, and hence these assays are called solid phase assays. Several solid phase systems are available and a widely used one is a coated tubes system [7, 11].

This system uses antibody-coated polystyrene plastic tubes into which the buffer, a standard or unknown one, and the tracer are added. After the incubation the contents of the tubes are discarded and the tube is rinsed to remove the last traces of the un-reacted reagents. The tube is then checked for radioactivity.

Material and methods

First we prepare a coating buffer. For this purpose we weigh 0.31 g of NaH_2PO_4, 2H20 and 1.2 g of Na_2HpO_4, $2H_2O$, also 0.5 g of NaN_3. Then we dissolve it in the distilled water and make one liter volume. After that we prepare the anti-PSA monoclonal antibody coating solution. The concentration of master monoclonal antibodies (mAb) is 7.5 mg/ml, and for making the coating solution we have to dilute it by one thousand times, that is up to 7.5 μg/ml. For obtaining this concentration we add 100 μl of the master mAb solution to 100 ml of the coating buffer. We mix it well gently and dispense 0.4 ml of this homogenous solution to each polystyrene tube, incubate the tubes overnight at the room temperature. Then we make a post-coating buffer as follows: 6.0 g. of Tris reagent, 3.2 g of Citric acid, 10 g of Sodium citrate, 1.0 g of NaN_3 (sodium azide), 10 g of BSA and 10 g of Sucrose, then we dissolve it in the distilled water and bring the volume to one liter.

The next day, we at first aspirate the antibody solution from the tubes then we dispense 0.5 ml of post-coating buffer to each tube and incubate it overnight.

During the coating process most of the antibody molecules are tightly fixed to the solid phase surface. But some of them, which are the low fixed antibody molecules, have to be removed during the post-coating treatment in order not to interfere with the future antigen-antibody reaction [9, 10]. At the same time, the final coated antibody molecules have to be protected during their conservation. For this purpose some protecting ingredients have already been added to the post-coating buffer as well. After the coating process the tubes must be dried in an appropriate room and the drying process lasts overnight. The coated tubes could also be kept in a humid form for a short term (for a few weeks). In this case, the coated tube could be kept with the post-coating buffer containing the amount of 0.1% NaN_3, at 4-8°C. The dried coated tubes are meant for a long-term conservation (from several months to years). For this purpose the dried coated tubes have to be packed in an appropriate box with desiccants.

We should also make the iodinated PSA-antibody (tracer) to perform the immunoradiometric assay (IRMA) with locally made anti-PSA coated tubes in order to evaluate the efficiency of our coated tubes. For this we use the other pair of anti-PSA monoclonal antibodies for iodination with radioisotope (^{125}I).

The best way for iodination of proteins or antibodies is the Chloramine-T method [1, 12]. First we take 100 μg (10 μl) of master monoclonal antibodies and pour them into a small plastic tube, then we add 10 μl (0.5 m Ci) of the iodine (^{125}I) solution and 10 μl (5 μg in phosphate buffer, pH = 7.4) of Chlorrmine-T which is an oxidizing agent and causes the conversion of negative iodine into positive iodine in order to bind the protein molecule. After 30 seconds we add 10 μl (5 μg in phosphate buffer, pH = 7.4) of Sodium meta bisulfite as a reducing agent to quench the reaction.

Then we purify the labeled mixture by using Sephadex G-25 column. Finally we dilute the labeled anti-PSA antibody (tracer) by phosphate buffer and adjust the activity of the tracer by measuring it in the gamma-counter to use it in the laboratory for doing the assay.

The assay protocol is as follows:

Patient sample serum and control serum standard is added sequentially to antibody-coated tubes 100 μl. 100 μl

of anti-PSA labeled antibody (tracer) is added to each tube and the tubes are mixed by Vortex mixer. Then the tubes are incubated at a room temperature for two hours while shaking.

At the end the contents of the tubes are aspirated carefully, the tubes are twice rinsed with 2 ml of wash buffer and aspirated immediately. Finally, the tubes are counted in the gamma-counter and the standard curve is made.

Results and discussion

After running the assay the results show a good and acceptable standard curve. The amount of PSA in the control serum and patient samples are compatible with the samples checked by Beckman's commercial kit (tab. 1, 2).

Table 1

The concentration of the standards and their counts

Standards	Concentration (ng/ml)	Counts per minute (cpm)
st. 1	0	360
st. 2	1	1264
st. 3	3	4962
st. 4	10	17321
st. 5	30	38678
st. 6	100	59864

Table 2

The comparison of the results between two different coated tubes

Patient Samples	Local kit PSA (ng/ml)	Commercial kit PSA (ng/ml)
1	4.6	4.7
2	2.2	2.1
3	5.9	5.8
4	1.9	2.0
5	3.6	3.5
6	7.5	7.6
7	8.3	8.2
8	12.4	12.2
9	9.6	9.7
10	4.2	4.1

As we see in fig. 1, the standard curve shows a good proportion between the radioactivity counts of the standard samples and their concentrations which is a typical IRMA curve.

The normal value of PSA concentration in healthy men is 0-4 ng/ml, but it is recommended that each laboratory should establish its own norm values for the local healthy population regarding the age and other potential ethnic and regional differences. When the sample value is higher than the concentration of the highest standard value, we should dilute it by a zero standard value. The mentioned thing usually

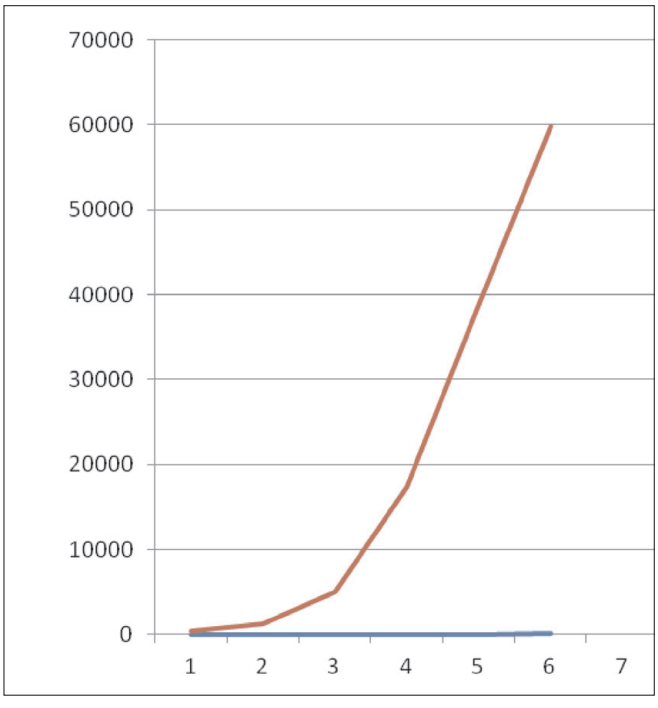

Fig. 1. The standard curve of PSA-IRMA using locally made anti-PSA monoclonal Ab coated tubes.

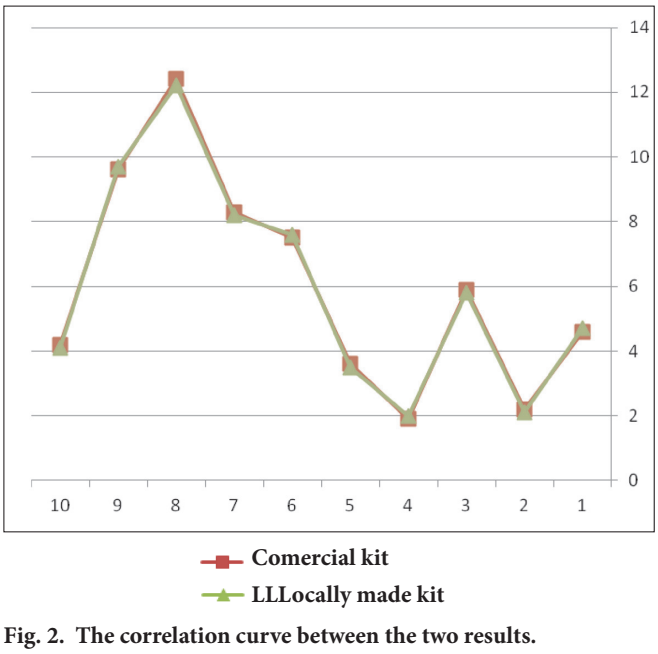

Fig. 2. The correlation curve between the two results.

happens to the samples of patients with developed prostate carcinoma accomplished by bone metastasis. In this situation the obtained results must be multiplied by the dilution factor.

After comparing the results of two different coated tubes we can easily come to the fact that there is a good correlation between the amounts of the PSA in samples measured by our local coated tubes and those which are made by means of the commercial kit (fig. 2).

Conclusions

Immunoassays are the most widely used analytical techniques and have been successfully applied to an extensive range of substances, including those with large and small molecules. Many solid phases have specific reactive groups which can be activated by a variety of biochemical methods. After introducing Yalow and Berson's isotopic method for measuring insulin in the clinical laboratories it has been spread very quickly to many analytes including the tumor markers. This research shows that we can manufacture the cost effective IRMA kit by producing antibody-coated tubes for PSA locally. It can also be used for the mass screening of prostate cancer in men over 50 in any country, including the developing countries thanks to the simple procedure and its reasonable cost. Finally, the same protocol for coating monoclonal antibodies on a solid phase can be used for the other tumor markers like CEA, CA15-3 and AFP if we have their specific monoclonal antibodies.

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Pulmonary embolism in acute varicose thrombophlebitis of lower limbs

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Abstract

Background: The purpose of the study is the improvement of treatment results in the patients with superficial vein thrombosis, associated with varicose veins (SVT-AVV) of lower limbs, through studying the frequency of pulmonary embolism (PE) cases and the factors predisposing to it.

Material and methods: 236 patients with SVT-AVV have been examined and the disease has been observed in 250 lower limbs. A triplex scanning of the venous system of both lower limbs with the registration of all embolic areas of superficial and deep veins has been carried out in every patient. PE clinic picture has been confirmed by computer angiopulmonography.

Results: The results of the study have showed that SVT-AVV is complicated by clinically distinct PE in 3.8% of cases (95% CI 1.8-7.1). The frequency of PE among the persons with SVT-AVV without a thrombotic process in deep venous system has amounted to 1.1% and when this process was present – to 14.6% ($p < 0,001$). PE has been registered more frequently in the patients with the thrombotic process in both lower limbs ($p = 0.004$). In the patients with SVT-AVV the frequency of PE has been significantly increasing along with the increase of the number of its possible sources ($p = 0.007$). The frequency of PE at SVT-AVV presence with the top clots localization in the small saphenous vein has not differed significantly from the same indicator of the great saphenous vein, indicating to the frequent underestimation of the role of this vein.

Conclusions: The survey of the patients with SVT-AVV should be directed to the thorough identification of all possible sources of PE, considering a special risk of its development in the persons with thrombotic process in both lower limbs and concomitant deep venous thrombosis.

Key words: venous thrombosis, varicose veins, pulmonary embolism.

Тромбоэмболия лёгочной артерии при остром варикотромбофлебите нижних конечностей

Введение

Острый варикотромбофлебит (ОВТФ) нижних конечностей часто считают безопасным и доброкачественным заболеванием, которое не является причиной летальных исходов [1]. На самом деле, летальные случаи при ОВТФ далеко не редкость, и обуславливаются они, главным образом, тромбоэмболией лёгочной артерии (ТЭЛА). В недавнем систематическом обзоре обнаружено, что при ОВТФ бессимптомная ТЭЛА регистрируется у 20-33% пациентов, а клинически выраженная – у 2-13% [2]. Исследование по оценке факторов риска развития венозного тромбоэмболизма (MEGA) обнаружило, что клинически выраженный ОВТФ связан с 3,9-кратным возрастанием риска ТЭЛА [3]. И даже при полноценной диагностике и стационарном лечении больных нередко встречаются смертельные исходы [4, 5].

Исходя из этого, важным при лечении ОВТФ является вопрос выявления той категории больных, у которых есть