in the abdominal lymph nodes – in children (57.2%), in the mediastinum – in children and patients aged 19-39 (48.2%).

4. Children develop only aggressive NHL variants, which also predominate in adults. Aggressive NHLs in adults have been predominantly diagnosed in the patients having the primary tumor focus location in the abdominal and mediastinal lymph nodes. The indolent NHL frequency has been higher in case of primary involvement of peripheral lymph nodes in patients aged over 60.

5. The frequency of metastases in the bone marrow and CNS has been dependent on the location of NHL primary focus, morphological variant and age.

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Functional recovery of retina after photo stress is accelerated by trans-cranial cerebellar stimulation in patients with diabetic retinopathy

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Abstract

Background: The characteristics of visual evoked potentials (VEP) have been studied in diabetic patients with and without diabetic retinopathy.

Material and methods: The magnetic impulses (2.0 Tl at the height of impulse) have been delivered to the cerebellar surface trans-cranially, using Neuro-MS/D, (Russian Federation), and the VEP have been registered every 20 seconds from the moment of the photo stress during one minute.

Results: An increase of the latency period and a reduction of the VEP amplitude have been recorded in the period following the exposure of the macular part of the retina to the photo stress. The VEP characteristics have been restored to the initial level at 74.3 $\pm$ 3.1 seconds from the photo stress moment in the control group, while in the diabetic patients with and without retinopathy this index has been 131.7 $\pm$ 10.2 and 86.5 $\pm$ 5.5 seconds respectively. In the presence of cerebellar trans-cranial magnetic stimulations (2.0 Tl, 40 impulses) the VEP amplitude depression has been less pronounced, and the restoration period of the VEP characteristics shortened to 90.5 $\pm$ 6.8 seconds in the patients with diabetic retinopathy.

Conclusions: 1. Retinopathy development is linked to a prolonged VEP latency period (P100), lowering N75-P100 amplitude, as well as to the enlargement of the recovery period of the retina's functional capacity in patients suffering from the diabetes mellitus in the presence of photo stress. 2. The cerebellar trans-cranial magnetic stimulation facilitates a quicker recovery of the retina's functional capacity in response to the photo stress in the diabetic patients with retinopathy. 3. The periodical cerebellar trans-cranial stimulations may be considered as a prevention method as well as a treatment method of diabetic retinopathy.

Key words: diabetic retinopathy, visual evoked potential, trans-cranial magnetic stimulation.

Introduction

Diabetic retinopathy is one of the causes of the development of the acquired blindness at the heart of which there are a microcirculatory bloodstream impairment, an increased vascular walls ductance and a vascularization development that lead to the retinal detachment [8, 10]. Retinopathy rarely develops in the first few years of the disease, but this pathology is observed in half of the patients who have been suffering from diabetes mellitus for 10 years, and in 90% of the diabetic patients with a 25-year clinical history. Due to the contemporary treatment methods of diabetes mellitus and an increased lifespan of diabetic patients, retinopathy has become one of the top-priority concerns among the other serious complications of the disease.

It has been lately established that retinopathy pathogenesis is linked to the activation of lipid peroxidation and to the increased concentration of pro-inflammatory cytokines in the retinal structures [10]. On the other hand, raising the neuronal brain tissues activity by means of electric stimulation promotes the brain's anti-oxidative potential [1] and can guarantee a neuroprotective effect, *inter alia* in the case of the ischemia-induced neurodegenerative alterations in the retina [7]. The objective of this research is to study the recovery process of the retina's functional activity in the patients suffering from diabetes mellitus following a photo stimulation (photo stress) test [12], as well as its special features under the conditions of cerebellar trans-cranial stimulation [3].

Material and methods

14 practically healthy individuals (the mean age 32.2 $\pm$ 3.7 years) and 27 insulin-dependent diabetics (the mean age – 31.2 $\pm$ 4.1 years) have been observed in this research.

All the subjects have provided a written consent to the research conduct. All investigations have been performed in accordance to the ethics requirements of the commission on ethics at Odessa National Medical University (Animal Care and Ethics Committee, 2008/84).

For selecting the control group the following criteria have been observed: the intraocular pressure less than 21 millimeters of mercury, a preserved acuity of vision, an unaltered visual field and an absence of eye diseases and neurological disorders. For the diabetics the criteria have been the following: the intraocular pressure less than 21 millimeters of mercury, a correctable acuity of vision (more than 7/10) as well as the absence of the signs of the proliferative retinopathy, which has been established by means of fluorangiography [12]. This method has allowed us dividing the diabetic patients into two groups according to the following classification [12]: the patients without retinopathy symptoms comprised the first group (11 patients); the patients with mild and severe proliferative retinopathy formed the second to fifth groups (16 patients). It should be noted that the acuity of vision in diabetic patients without retinopathy has been preserved (10/10).

While conducting the research the patients were being observed in a dark and soundproof room. Before recording the visually evoked potential (VEP) each subject was adapting to the light intensity for 10 minutes until the moment when the pupil diameter became equal to 3 mm. The level of the background screen glow was 5 cd/m^2.

VEP has been recorded using the adopted technology [2, 12]. The visual stimuli – chess pattern – has had the contrast degree of 70% and the mean luminescence degree of 110 cd/m^2, while the contrast reversion has been carried out with a frequency of two times per second. A subject has been placed at a distance of 114 cm from the screen center what has allowed a 15-seconds acceptance angle of separate pattern elements and a 25^o acceptance angle of the screen height. Only the right eye of all the patients has been tested while the left one remained closed [2, 12].

The electrodes have been placed at points Oz (the active electrode) and Fpz (the reference (recording) electrode); the grounding has been fixed on the left arm. The inter electrode resistance has been set at 3 kOhm. The bioelectric signals have been amplified by 20 000 times; the signal transmission band has comprised 1-100 hz, after that the signals have been averaged, and the artifacts have been eliminated for the stimulation period [2]. The experiment has been organized in the form of not less than two sessions of stimuli exposure; during each one 100 answers have been averaged after the artifacts elimination. The VEP analysis period has been 500 ms. The visual VEP in this case has included several waves, among which three peaks always could be distinguished that normally appeared in 75-100 ms and 145 ms. These peaks have had negative (N75), positive (P100) and negative (N145) polarities (fig. 1).

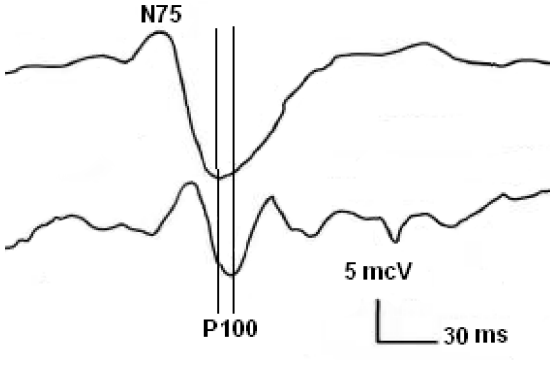

Fig. 1. An induced visual potential in practically healthy patients (the upper curve) and in the patients with diabetic retinopathy (the lower curve). A lengthening of the latency period P100 can be observed – the distance between the two vertical lines. A lowering of the potential amplitude N75-P100 is also visible.

After the preliminary testing the control VEP have been recorded while 40 stimuli responses have been averaged. These recordings served as a basic control of the VEP characteristics. A 30-second-long photo stress has been induced using the 200 W candescent lamp that has been placed at a 20 cm distance from the eyeball and has been projected onto the retina as a visual field loss with a 6-grades diameter. As a result, the pupil diameter has narrowed up to 2 mm. Immediately after the photo stress exposure VEPs have been recorded and the answers have been averaged every 20 s sequentially until the moment the resulting answer becomes identical to the basic

control record. The time period till the moment of the VEP characteristics recovery has been recorded as the recovery time after the photo stress.

The device Neuro-MS/D, (Neurosoft, Russian Federation) has been used for the trans-cranial magnetic stimulation along with the appropriate coils and 2.0 Tl induction at the height of the magnetic-influence impulse. The coil has been positioned on the middle line of the occipital zone in the tangential plane to the cranium surface according to the adopted cerebellum stimulation technique [6]. In the control group the false stimulation has been carried out positioning the coil perpendicularly to the same occipital zone [9].

The research results have been statistically processed applying the ANOVA method and the Newman-Keuls test.

Results and discussion

Dynamics of the development of the latency period (LP) P100 has revealed that before the photo stress exposure onset in the group of patients with diabetic retinopathy this index exceeded that in the control group by 27.0% ($p < 0.05$) (fig. 2, A). At the same time, the VEP LP in the group of patients without retinopathy has been by 13.9% ($p > 0.05$) higher compared to the control group as well. In the first 20 s following the stress period, the LP in the group of patients with diabetic retinopathy has exceeded that in the control group by 33.5% ($p < 0.05$) and has been higher than that before the photo stress exposure by 17.5% ($p > 0.05$). A significant difference from the control group has persisted until the end of the observation. Meanwhile, the LP recovery to the basic value has been recorded in the control group after 74.3 ± 3.1 s, in the group of patients without retinopathy – after 86.5 ± 5.5 s, and in the group of patients with retinopathy – after 131.7 ± 10.2 s (fig. 2, V).

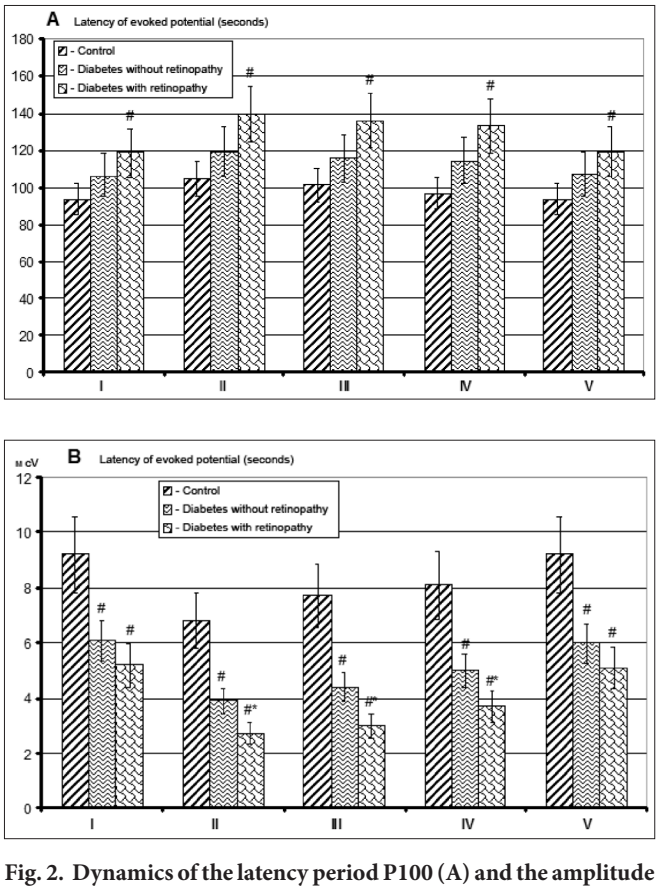

Fig. 2. Dynamics of the latency period P100 (A) and the amplitude N75-P100 (B) after the photo stress.

Legend: abscissa I – before starting the photo stress; II – 20 s; III – 40 s; IV – 60 s after photo stress exposure; V – complete recovery of the studied indices. Ordinate – the studied indices (seconds and mcV). # – $p < 0.05$ in comparison to the control group index; * – $p < 0.05$ in comparison to the index in the group of patients without retinopathy (ANOVA+ Newman-Keuls test).

The amplitude N75-P100 has been by 33.7% ($p < 0.05$) lower in patients without retinopathy than in practically healthy patients (fig. 2, B). In the group of patients with retinopathy such differences comprised 43.5% ($p < 0.05$). For the first 20 s of post-stress exposure 26.1% ($p < 0.05$) lowering of the amplitude N75-P100 has been observed in comparison to the initial background in the control group, while in the groups of patients with and without retinopathy it has been 48.1% ($p < 0.05$) and 36.1% respectively. At the same time, 30.8% ($p < 0.05$) decrease of the studied index has been recorded in the group of patients with retinopathy compared to that in the patients without retinopathy (fig. 2, B, II). The significant differences between the groups have persisted for 60 s of the post-stress observation (fig. 2, B). Moreover, in the 40 s of post-stress period the amplitude N75-P100 has been lower by 34.8% in the patients without retinopathy than that in the control group, and in the group with retinopathy – by 44.6% ($p < 0.05$). The full recovery of the studied index – the amplitude N75-P100 – has been observed during the same time period as the latency period of P100 recovery.

The effects of trans-cranial magnetic stimulation (TMS) influence upon VEP characteristics have been investigated in the group of patients suffering from diabetic retinopathy (16 patients).

The exposure to the photo stress under the conditions of the preceding TMS application (10 impulses) has been accompanied with a tendency of the latency period of P100 shortening, which has been by 5.0% ($p > 0.05$) shorter in the first 20 s of the post-stress exposure than in the group of patients with diabetic retinopathy in the absence of the TMS (fig. 3). This index has been relatively lower in the patients who have been exposed to the 40 impulses TMS during an analogous time period from the moment of the photo stress exposure, namely by 33.8% ($p < 0.05$), and it has been by 15.2% ($p > 0.05$) higher than in practically healthy individuals. Such differences between the observed groups have persisted for 60 s from the moment of photo stress exposure (fig. 3).

The VEP amplitude in the group of patients with diabetic retinopathy, who have undergone 10 impulses cerebellar TMS exposure during the first 20 s from the moment of the photo stress exposure, has been by 13.1% ($p > 0.05$) larger than in the patients of the given group in the absence of the TMS application (fig. 4). In the subgroup of patients with a larger number of TMS impulses (40 impulses) during an analogous time period the studied index has been by 17.8% ($p < 0.05$) higher than in the patients without TMS, but still it has been lower by 5.7% ($p > 0.05$) compared with the respective index

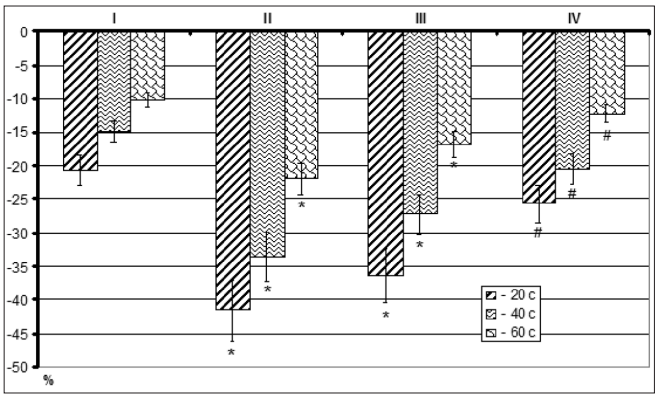

Fig. 3. Dynamics of changes of the latency period P100 in the photo stress test under the conditions of cerebellar trans-cranial stimulation application.

Legend: abscissa I – control group (practically healthy individuals); II – patients with diabetes and retinopathy; III – effect of 10 cerebellar TMS impulses; IV – effect of 40 cerebellar TMS impulses.

Ordinate – investigated indices in % pertained to their corresponing levels taken as 100% which have been the following: for the control group (I) – data on the VEP without photo stress has been taken as 100%; for group II – photo stress in patients with diabetes retinopathy without TMS has been taken as 100%; for groups III and IV – photo stress in patients with diabetes retinopathy without TMS has been taken as 100%.

*-$p < 0.05$ – in comparison to the control group; #-$p < 0.05$ – in comparison to the index registered in group II – photo stress in patients with diabetes retinopathy without TMS (ANOVA+ Newman-Keuls test).

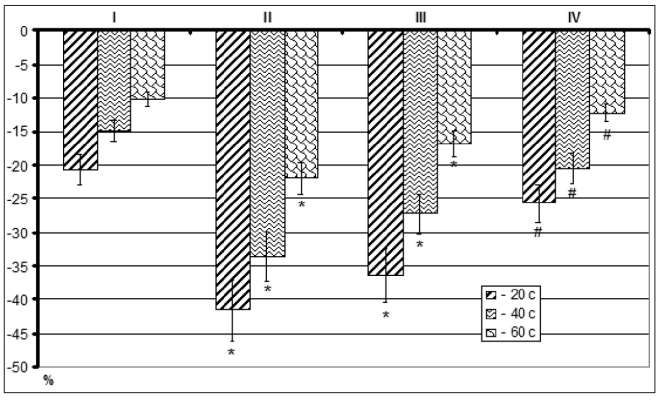

Fig. 4. Dynamics of the changes of the amplitude of N75-P100 (B) in the photo stress test under the conditions of cerebellar trans-cranial stimulation application.

Legend: the same as in fig. 3. Sign (-) means magnitude of reduction of VEP amplitude compared with the data registered before the photo stress in the healthy persons (group I) and the patients with diabetes (groups II-IV), retinopathy taken as 100%.

*-$p < 0.05$ – in comparison to the control group; #-$p < 0.05$ – in comparison to the index registered in group II – photo stress in the patients with diabetes retinopathy without TMS (ANOVA+ Newman-Keuls test).

in the control group. In the last third of the VEP recording period the amplitude N75-P100 in the group that has been exposed to 10 impulses exceeded its value in the control group by 7.3% ($p < 0.05$), and in the group with 40 impulses application – by 2.1% ($p > 0.05$).

The recovery period of the studied indices in the group of patients with diabetic retinopathy comprised 90.5 ± 6.8 s, while in the patients without retinopathy this index comprised 75.4 ± 5.9 s ($p > 0.05$).

Thus, the obtained results have showed that in patients suffering from the diabetes mellitus both with and without retinopathy there are the following impairments of the VEP characteristics observed: lengthening of the latency period of P100 and lowering the amplitude N75-P100 in comparison to the indices in the control group. Furthermore, the significant changes of the latency period have been recorded only in the patients with diabetic retinopathy.

It may be assumed that the described disorders are the result of a decreased velocity of the nerve impulse conductance along the optic nerve [5]. This hypothesis corresponds to the research results [5, 10] that show a reduction of the electroretinogram amplitude pattern in the absence of the retinopathy signs, which can be explained by a selective damage of the ganglion cells and their axons in the central part of the retina – by a continuous hyperglycemia. At the same time, a positive correlative relation between VEP changes, peripheral diabetic neuropathy, reduction of the central velocity of the signal conductance and histological disorders has been established [12]. As soon as the development of the optic nerve neuropathy has been shown as a result of the ganglion cells dysfunction [5, 10], the role of retinopathy in the established VEP changes cannot be excluded. Another fact speaks in favor of this assumption, namely, that the latency period of P100 in the patients with a proliferative retinopathy has been to some extent longer in comparison to that recorded in the absence of retinopathy.

The information regarding the functional state of retina has been obtained under the conditions of photo stress exposure. In the control group the light stimulation of the macular part of the retina has increased the latency period and lowered the VEP amplitude, while the VEP recovery period has been recorded during the period up to 80 s from the photo stress completion. It may be assumed that the photo stress impairs the ability of the macular photoreceptors to produce the action potential after such stimulation. Meanwhile, a prominent role in the VEP recovery seemingly belongs to the photo pigment re-synthesis that can be supplied by a sufficient bloodstream volume [12]. A positive TMS influence, thus, can be explained by the change of metabolism in the retinal tissue that appears, probably, due to the antidromic excitation conduction along the bisynaptic retinal-cerebellar path [4]. Also rather probable is the effect that develops due to the general metabolic TMS effects, which induce alterations on the part of the neurotransmitter systems in different brain structures [1, 11].

It should be also noted that the VEP after the photo stress in the patients with diabetes and without retinopathy, who have preserved vision acuity, and in the absence of the changes at fluoroangiography, can indicate the presence of the early functional incompetence of the central retinal layers [12]. The positive cerebellar TMS effects may point at the possibility of this method application for the purpose of prevention of the progressive development of neurodegenerative alterations on the part of retina in patients suffering from diabetes mellitus.

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,