ASSESSMENT OF THE TROPICAMIDE CONCENTRATION ON PUPILLARY RESPONSE TEST FOR ALZHEIMER'S DISEASE

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Abstract— **Pupillary response test using a cholinergic antagonist, tropicamide was focused as a diagnostic method of Alzheimer's disease (AD). Ever since the test was reported, there have been studies that non-AD individuals showed same responses to tropicamide compared to AD individuals and that the test could not be useful for the diagnosis of AD. In this study, we changed the experimental conditions especially the tropicamide concentration, and proposed the new test under this condition. We made tropicamide half the concentration of the original test and performed the pupil test for non-AD subjects. We obtained the results that non-AD patients could not be hypersensitive to the new concentration of the eye drops. This could be helpful for the test to reduce the false positive rates.**

Keywords— **Alzheimer's Disease, Vascular dementia, Pupillary response test, Tropicamide**

I. Introduction

The number of dementia patients is gradually increasing due to increase of the average human life span. Not only the patients' lifestyle but also their families' could change drastically. The disease starts with debilitating memory and dysfunction of recognition. Dementia can be classified into two categories by its origin and pathology. One is vascular dementia (VD) which is caused by vascular disorder, and the other is Alzheimer's disease (AD) which is caused by degenerative origin. Many VD patients have hypertension and arteriosclerosis, and multiple infarctions may be observed in their brains. This dementia can be cured with medication such as drugs to improve blood flow and is categorized as treatable dementia. On the other hand, AD is a neurodegenerative disorder of the central nervous system. This disease is characterized by progressive memory loss and the decline of other higher cognitive functions such as attention.

There is no way to stop the progression of AD yet. Although the two types of dementia have different onsets and courses, it is hard to distinguish the two in the early stages. Recently, diagnostic imaging such as MRI and SPECT, and biochemical tests are performed on the patients. In addition, mental and psychological tests are given to the patients along with interviews of family members. Screening can be done by obtaining this kind of information. The type of dementia can only be determined after the symptoms appear in mid term. However, if differential diagnosis could be done in the early stages, it could be possible to determine treatment methods and medications for reducing the disease.

Even though it is very important for dementia diagnosis to differentiate the type of dementia at an early stage, there is no neurobiological test for simple and accurate diagnosis of AD in the early stages. In 1994 Scinto et al. reported that patients diagnosed with probable AD by standard clinical criteria exhibited a marked hypersensitivity in their pupil dilation response to a cholinergic antagonist, tropicamide, placed in their eyes [1]. AD patients have typical brain lesions. The lesions, which are caused by intracellular neurofibrillary tangles composed of abnormally phosphorylated cytoskeletal proteins and complex protein deposits called amyloid, are found in the cerebral cortex. Neurobiochemical dysfunction related to disorder of the cholinergic and adrenergic systems have the characteristics of AD. Tropicamide is administered to the eyes when ophthalmoscopy is performed. It blocks the cholinergic receptor sites of the sphincter muscle of the pupil.

Scinto et al. used very dilute tropicamide (0.01 %) in three groups: (i) subjects with probable AD, with suspected AD and classified as cognitively abnormal, (ii) subjects with other dementia, and (iii) elderly controls. They found that group (i) showed significant difference from groups (ii) and (iii). Group (i) had greater pupil dilation than other groups. They used the following method. A single drop of a very dilute tropicamide solution was administrated to one eye and a drop of sterile water to the other eye. After application of the tropicamide, pupil diameter was measured seven times over the course of 1 hour for 30 s at 2, 8, 15, 22, 29, 41, and 51 minutes. They determined that the cutoff point of change in pupil diameter between group (i) and the other groups was 13 % at minute 29 of the assay [1].

After that, many studies were conducted all over the world and reported the results of the reviews. Kardon summarized several 19 reports about the pupil reaction to the drops [2]. Those studies dealt with 392 AD patients and 498 healthy control subjects. The report said that all 19 studies except the pilot study of Scinto showed that the pupil of the subjects with AD was hypersensitive to the drop and other subjects such as non-AD or control subjects also had the hypersensitivity. That could mean the test might be not able to detect AD patients from others. He said that all studies except Scinto et al. showed that the pupil test could not be a clinically useful diagnostic test for AD. However, Scinto et al. contrarily said that they did not propose clinical use of the pupillary test. They said that an exaggerated dilation response to dilute tropicamide might serve as a potential diagnostic marker for AD [3].

In our study, we first used 0.01 % tropicamide, which Scinto et al. used in their pilot study and the other researchers also used. We found in our first study that the

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Abstract

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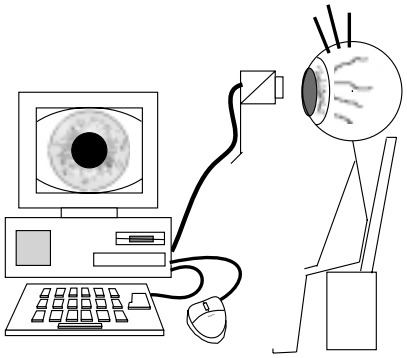

Fig. 1. Experimental system

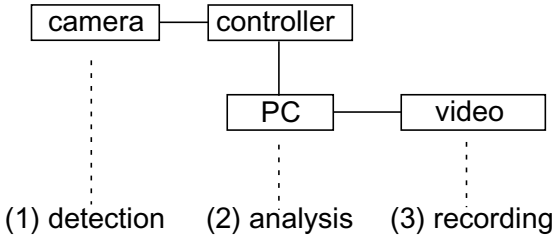

Fig. 2. Construction of the system

subjects with AD reacted to tropicamide and showed high dilation of the pupil. That result was identical to the pilot study of Scinto. However non-AD subjects (VD patients and control) also showed same dilation, even though Scinto et al. said non-AD subjects had no pupil dilation. Our results indicated an insignificant difference in pupil dilation between AD and non-AD subjects. Our results indicated that it was difficult to differentiate AD from other dementia patients using this method and the criteria that Scinto et al. demonstrated. In this study, we used two different concentrations of tropicamide, one of which was dissimilar to that of the pilot study, and applied this to the group of VD patients reacting to the previous concentration of tropicamide. The rates of the pupil dilation between two groups were compared in order to confirm the different trend of pupil dilation. In order to detect pupil area precisely, an original pupilometer system was developed. This system contained an infrared CCD camera and a PC for the analysis of pupil area. It used video images and computer image processing for the quantitative calculation of pupil dilation rate.

II. Methods

In our study, we used 0.01 % and 0.005 % tropicamide. The test for confirmation of pupil dilation in response to tropicamide was done as follows. (1) Subjects with VD were instilled with 0.01 % tropicamide. (2) Subjects with VD also were instilled with 0.005 % tropicamide, half the concentration of the first test. Diagnosis of dementia was done with MRI and SPECT images and based on DSM-R-IV. Subjects were seated in an experimental room with controlled illumination. After scotopic adaptation for about 5 minutes, one drop of tropicamide solution was

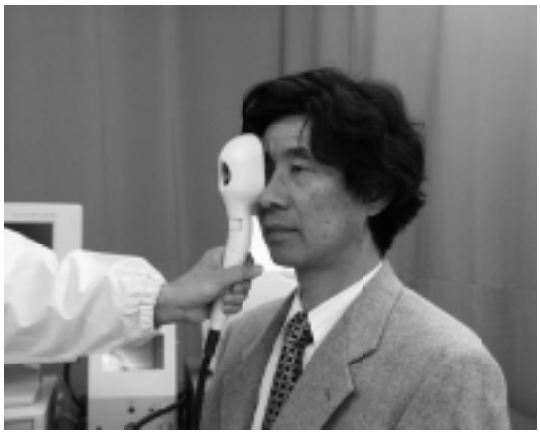

Fig. 3. Detection of eye image

administered to one eye and one drop of saline solution to the other eye.

Images of both eyes were obtained before instillation, as initial eye area, and every 5 minutes up to 1 hour after application of tropicamide. The subjects watched an object in front of them in order to fix their focus during eye image detection. The distance between their eyes and the object was 1m. During the eye drop test, the dementia subjects were tested on their mental and memorizing functions. The test was HDS-R (Hasegawa Dementia Scale-Revision), which is widely used in Japan. The full score is 30 and they were scored by their replies. Patients with scores under 20 can be diagnosed as dementia. The questions in HDS-R are mainly about memorizing functions. In this study, we chose subjects with same degree of dementia by the HDS-R score. All subjects were suffering from VD, who had the typical lesion of vascular disorders in their brains. We dealt with very early stage subjects with HDS-R scores over 20.

III. System

An image analysis system containing a video and a PC was developed for the calculation of pupil area. Fig. 1 shows the experimental system. The system consists of (1) detection, (2) analysis and (3) recording devices (Fig. 2). The detection part (1) contains an infrared CCD camera, illuminating infrared LEDs, and a half mirror (Fig. 3). In order to reduce the LED reflection on the cornea, polarizing plates are set in the device. For sufficient light intensity and eye safety, the LEDs are illuminated on pulse. In the analysis part (2), image analysis was performed as follows. (a) The images were captured on a PC by an image control board. (b) The captured images were converted into binary images with the pupil in black and the other parts of the eye in white. Each captured image was stored in the PC memory and the coordinates and gray level were calculated for each pixel in the image. A gray scale histogram was created. From the histogram, a threshold dividing the black and white was calculated automatically. The iris area and the pupil area were extracted using the threshold. Even if pupil size changes

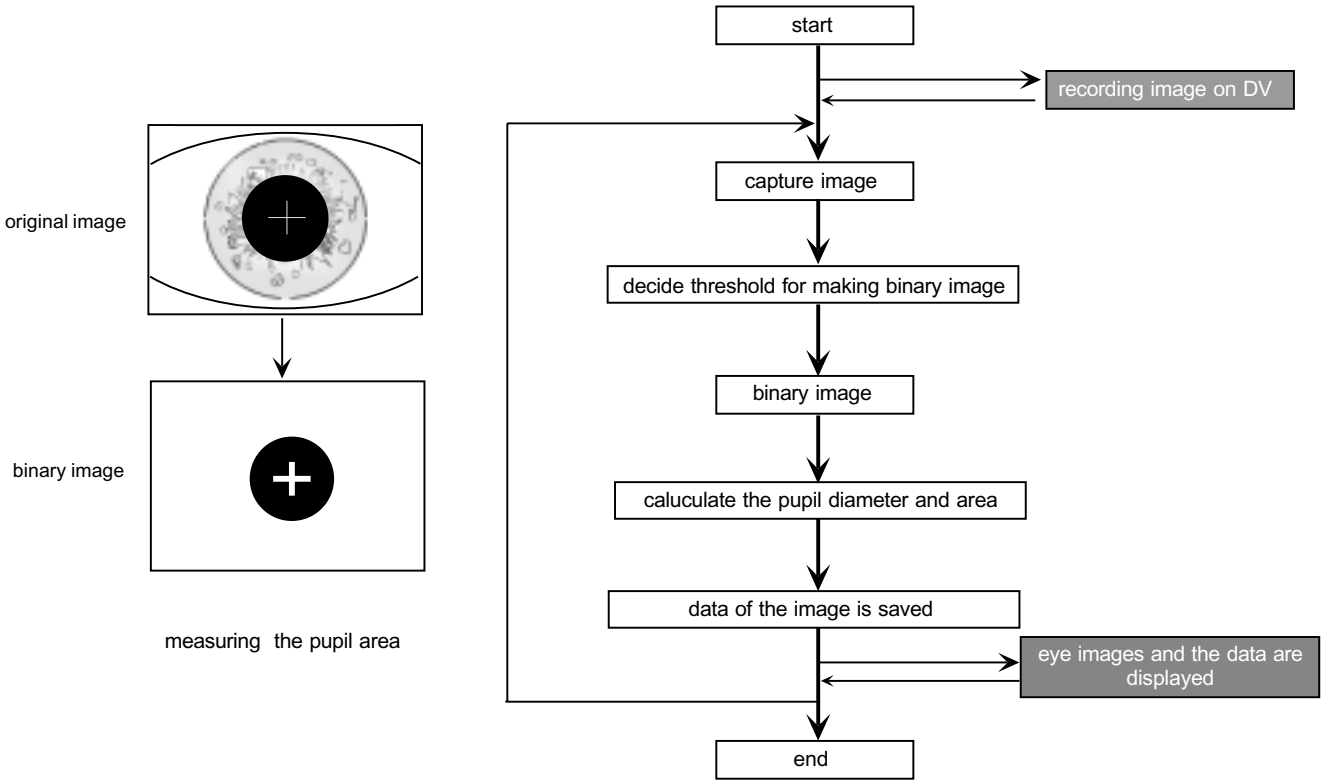

Fig. 4. Flow chart

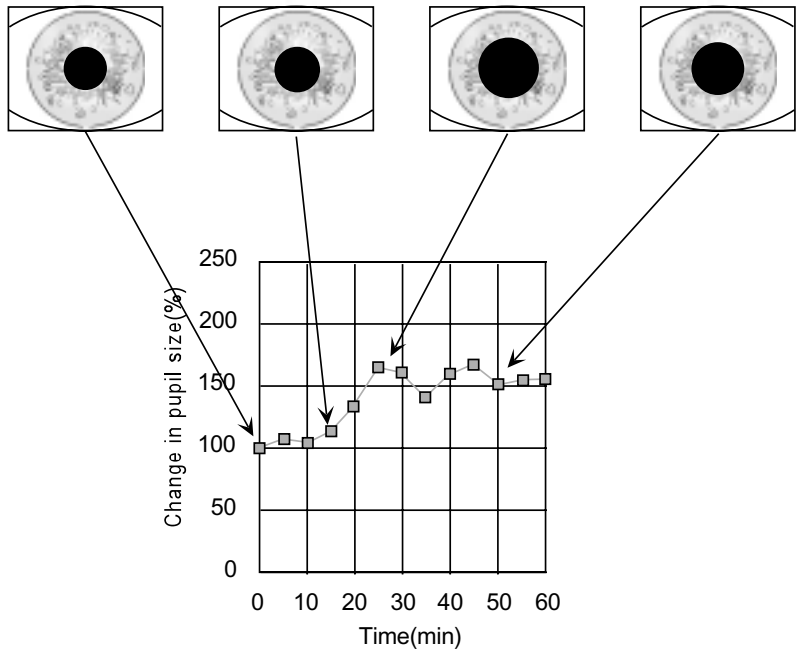

Fig. 5. Sample

continually, the iris area is constant. So the variations of pupil size were calculated based on the iris diameter as a reference. Fig. 4 shows the chart of the analysis. The recording part (3) contains a video recorder. Captured images were simultaneously recorded on digital video for review. The results of the pupillary test were displayed on a graph where the horizontal axis is time (min) and the vertical axis is change in pupil size (%). Initial pupil area was expressed as 100 % and this value was used as a reference for change in pupil size. The shape of the line was the trend of the pupil dilation (Fig. 5).

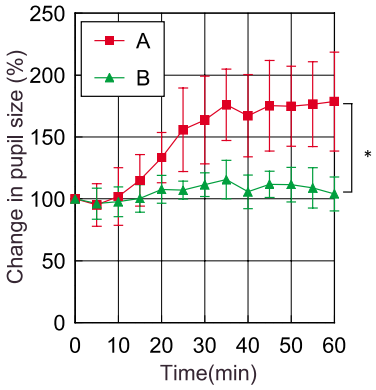

Fig. 6. Comparison of the mean dilation trends
* $P < 0.001$ (Multiple comparison; Turkey test)

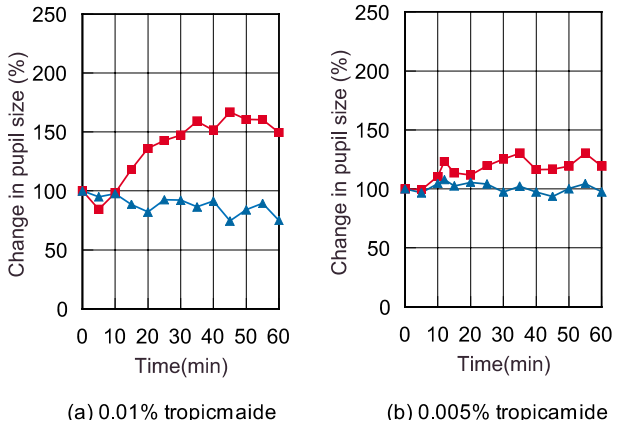

Fig. 7. Difference on the concentration of tropicamide

IV. Results

Fig. 6 shows the trend of pupil dilation for 0.01 % and 0.005 % tropicamide for 60 minutes after application. This figure has two lines, (A); indicating the average pupil size of VD subjects with 0.01 % (n=10) and (B); with 0.005 % (n=10). Multiple comparisons were performed with a Turkey test between the two groups of subjects. There was significant difference between the two groups.

Fig. 7 shows the results of the different concentrations applied to one subject with VD. Fig. 7(a) shows the change in both pupil areas (application of 0.01 % and control side) respectively and Fig. 7(b) also shows the dilation trends with 0.005 % and control.

V. Discussions

In the test using 0.01 % tropicamide, large pupil dilation were found in VD subjects. Therefore the test under these conditions could not be efficient as a screening method for dementia patients. VD subjects who were not AD were hypersensitive to 0.01 % tropicamide. Using this method happened to increase the false positive rate of AD. Using 0.01 % tropicamide, VD patients could be recognized as AD . When 0.005 % tropicamide was applied, VD subjects had no pupil dilation. This result supported the claim of Scinto et al. that non-AD patients exhibited less hypersensitivity in their pupil dilation response to a cholinergic antagonist.

The pupillary response test has many merits. It is very simple, noninvasive and harmless. Tropicamide is a safe drug used in an ophthalmic test. Since pupil size can easily change due to physiological conditions and to external environment, the pupil size is measured at 5 minute intervals over a 60 minute period and the average pupil dilation rate is calculated. Calculating the average can reduce the effect of sudden change of pupil size.

In our study, we developed an image analysis system and used it for the precise detection and analysis of the pupillary behavior. Our system has a very compact probe as shown in Fig. 3. Users can handle the system with ease. Patients can just sit and the eye images are taken with no contact methods. Therefore the detecting action is done only when the images are taken. Therefore the procedure gives the patients no irritation.

The test takes 60 minutes in total. It might seem a little long but it is necessary to take 20〜30 minutes for the eye drops to sufficiently be absorbed into the eyes and after that, exact pupil response can be observed.

In this study, we focused on the phenomenon that Scinto et al. demonstrated that only AD subjects have hypersensitivity to tropicamide. Tropicamide with different concentrations were instilled to VD patients and we found that 0.005 % tropicamide cannot affect non-AD patients. The result indicated that the dilute concentration could be useful for the drop test to differentiate two types of dementia. In our further studies, we will continue to perform the pupillary test for AD, VD and younger control subjects and will compare the dilation trends of the three groups. The results of our further studies will be helpful for the pupillary response test as a screening method of dementia.

VI. Conclusion

We used two different concentrations of tropicamide (0.01 % and 0.005 %), which were instilled to VD patients groups. The change in pupil size with 0.01 % was larger than with 0.005 % and there was a significant difference between the two groups. It could be very useful for the screening test in dementia to apply 0.005 % tropicamide. It could be probable that the false positive rates could be lowered because non-AD subjects such as VD individuals don't react to 0.005 % tropicamide. This study could promote the realization of the pupillary response test for the diagnosis of dementia.

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