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Learning Effect and Test-Retest Variability in Healthy Subjects and Patients with Primary Open Angle Glaucoma Using Rarebit Perimetry

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Abstract: *Aim: To study learning effect (LE) and test retest variability (TRV) in healthy subjects and patients with primary open angle glaucoma (POAG) using Rarebit perimetry (RBP). To determine normative ranges of RBP.*

Methods*: 61 eyes of 35 subjects underwent visual field testing with standard automated perimetry (SAP) and RBP. TRV and LE were assessed in repeated examinations conducted in 3 different days. First two examinations were conducted within 3 days and the last one within one month. LE was assessed by comparing results from the three sessions. TRV was evaluated by calculating differences between retest for each combination of single tests. To determine normative ranges of RBP were included 34 eyes of 21 healthy subjects and 62 eyes of 47 subjects with preperimetric and early POAG. Cut off value was determined between the two groups using ROC analysis.*

Results*: No significant LE was observed in POAG group. There was a significant LE in the control group but only in the visual field zones with eccentric location. TRV was higher in POAG group and in central visual field zone. The mean MHR in control group was 94.88 (SD 2.21) and 83.56 (SD 6.95) in POAG group. Cut off value for discriminating between healthy subjects and patient with POAG was 91.50% with AUROC 0.985 ($p<0.001$, ROC analysis).*

Conclusion*: RBP is fast and easy to perform test. RBP testing did not show a significant LE in glaucoma group, however, TRV was consistent. MHR can be successfully used for differentiation of healthy eye from those with early glaucoma changes.*

Keywords: *learning effect, normative ranges, rarebit perimetry, test-retest variability*

I. Introduction

The main indicator that is used to diagnose glaucoma is visual field changes.

Standard automatic perimetry (SAP) is the preferred method for diagnosis and follow up of glaucoma, but studies have shown that in order to establish a defect in the visual field of glaucoma ill patient at least 30% of the ganglion cells should be dead [1]. That is why structural changes occur before functional on SAP in 60% of patients according to data from OHTS [2]. Low sensitivity of SAP can be attributed to several reasons.

One is the amount of information which is contained in the test stimuli [3]. Test targets in SAP are large enough so they cover several receptive fields and still be detected even with partially damaged retino-cortical neural channels. The other is high test-retest variability (TRV) and learning effect (LE) which are broadly discussed for SAP [4] but practically characteristic for any visual field test.

Variability in the results is an inherent feature of any perimetric examination. It is due to factors such as fatigue, LE, visual artifacts and is generally based on psycho-physiological grounds [5]. The effect of training is reported in numerous studies of various perimetric tests and is manifested as improvement in sensitivity, reduction of variability and better understanding of the nature of the test procedure [6]. Variability in retest has been observed both within the same test and in successive tests. It is believed that it is greater in eyes with glaucoma than in healthy [7] and correlates positively with the depth and size of the defect [8] although there are contradictory opinions [9]. The most significant effect of learning is observed during the second to the third perimetric study [10].

Rarebit (microdot) perimetry (RBP) was developed by Frisén [11]. This is a relatively new technology which addresses all reasons for low sensitivity of SAP described above. It uses a standard LCD monitor to present very small stimulus (dots) on a dark background. The dots are presented in pairs. The patient must answer for each presentation how many dots does he sees: one, two or none. Instead of measuring the threshold of sensitivity to light, the test used microdots to assess the integrity of retinal architecture and retino-cortical neural channels. Changes in the field are presented as clusters of micro defects in the system - as "micro holes on a flat surface". These micro defects are accumulated in a larger amount within the established visual field

defects, and the more profound defect, the greater the density of "micro holes". Rarebit visual field defects have spatial distribution similar to that of SAP, but the defects with sloping boundaries appear larger.

The purpose of our study was to determine whether RBP can discriminate between healthy eyes and eyes whit early glaucomatous changes and to examine thoroughly the learning effect and test–retest variability of this procedure.

II. Methods

The clinical research took place over a period of one and a half year – from March 2015 to August 2016. The selection of patients and all necessary tests were conducted in the Ophthalmology department at University Hospital "Aleksandrovska".

The study followed the tenets of the Declaration of Helsinki and informed consent was obtained from all participants. The study included 31 eyes of 16 healthy subjects and 30 eyes of 19 patients with POAG who underwent threefold examination with RBP in order to determine TRV and LE and in order to determine normative ranges of RBP were included 34 eyes of 21 healthy subjects and 62 eyes of 47 subjects with preperimetric and early POAG. Included subjects were Caucasians, aged 30 to 75 years. All subjects underwent an ophthalmological examination (including best corrected visual acuity (BCVA) evaluation, Goldmann applanation tonometry, gonioscopy, slit lamp examination, fundus biomicroscopy. Visual field examination was performed with SAP (Humphrey field analyser (HFA) II; Carl Zeiss Inc., Dublin, CA) and RBP (software version 4). All participants in the study were examined with optical coherent tomography (OCT) - 3D OCT 2000 Topcon during moderate pharmacological mydriasis.

Inclusion criteria were BCVA≥0.7 (represented in decimal visual acuity values), open anterior chamber angle, absence of any other ocular pathology. Exclusion criteria included ametropia>3dpt, pupilar diameter<3mm, alterations in anterior chamber angle or other conditions that may cause secondary glaucoma, other ocular or systemic pathology that may alter the visual field.

SAP testing was performed with 24-2 program, Swedish Interactive Threshold Algorithm (SITA) Standard strategy (HFA II; Carl Zeiss Inc., Dublin, CA). Only reliable SAP results were considered, defined as false-possitive and false-negative responses <33%, and fixation loses <20%.

Visual field tests were classified as glaucomatous according to Hoddap-Parish-Anderson criteria: glaucoma hemifield test outside normal limits or a cluster of three or more non-edge points in a typical location of glaucoma, all depressed on the pattern deviation plot at a p<5% level and one depressed at a p<1% level or a PSD that occurs in less than 5% of normal visual fields.

The results on SAP are classified according to the criteria of EGS (version 4) and Glaucoma staging system [12]. The system proposed by Mills et al. has six stages. Stages 0 and 1 were considered, which included tests having a mean deviation (MD) to -6dB.

All patients and controls were examined with OCT (3D OCT 2000 Topcon) – 3D Macula (V) and 3D Disc protocols. Only pictures with good quality (index>30) were considered.

The patients were classified into three groups:

- control group: eyes with normal visual field on SAP and MD <-2 dB, without damage to the optic nerve and intraocular pressure (IOP) <21 mmHg (double measurement within 3 days)
- preperimetric glaucoma group: characterized by diffuse or localized thinning of neuroretinal rim, diffuse or localized defect in nerve fiber layer (RNFL), normal visual field on SAP, MD <-2 dB and IOP> 21 mmHg
- preperimetric glaucoma group: IOP> 21 mmHg, glaucomatous thinning of neuroretinal rim and typical glaucoma visual field defect

Participants were examined with RBP (central 30° test, software version 4). RBP procedure has been described in detail elsewhere [11]. In brief, microdot targets are presented within 24 separate rectangular test areas inside 30° of eccentricity. The test target is composed of two microdots, having a diameter set at half of the minimum angle of resolution (approximately 1/100 the size of the SAP stimulus), separated by 4˚ of the visual angle and simultaneously exposed. For each test area, four passes of stimuli were performed in ever new positions. A fixation mark is moved at in a preset sequence by the computer. Subjects are instructed to fixate it on the monitor, to indicate the number of microdots seen during each presentation (0, 1, or 2) by not clicking, clicking, or double clicking a mouse button. In accordance with the developer's recommendation, a Thin Film Transistor (TFT) monitor was used for RB testing at an analysis of 1024x768 pixels. Default settings were used for contrast, brightness and target duration. The test was first performed at 0.5 metres of distance, to test the peripheral areas, and repeated at a distance of 1 metre, for the central locations. Prior to the RBP testing, a demonstration was run to ensure that the task was understood and the subject could respond to the stimuli in an appropriate manner. The proper refractive correction was used in each case.

The RBP test results are shown as a percentage hit rate, defined as the total number of dots seen divided by the total number of dots shown. The printout provides an mean hit rate (MHR) and standard

deviation (SD) (MHR-SD), representing an average of all tested areas (except for the one closest to the blind spot). Mean miss rates (MMRs) are also provided for each of the 24 tested areas in the form of a percentage (shown as 0%–100% in 12% increments, when four RBP runs were completed) and graphically with a gray-scale plot. The error statistic represents the sum of the responses to control presentations, which should be close to 0. Only reliable RBP tests, defined as having an error statistic of <2 were considered.

The parameters analyzed in our study included:

From SAP: MD, PSD

From RBP: Parameters MHR, MHR-SD and number of tested areas with hit rates <90% from the three consecutive test were used to evaluate TRV and LE. In examining the influence of the eccentricity on the RBP parameters, the tested areas were divided in three zones (Fig. 1): central (4 central areas), midperipheral (7 areas around the central ones), and peripheral (remaining 12 external areas). To determine normative ranges of RBP was used MHR.

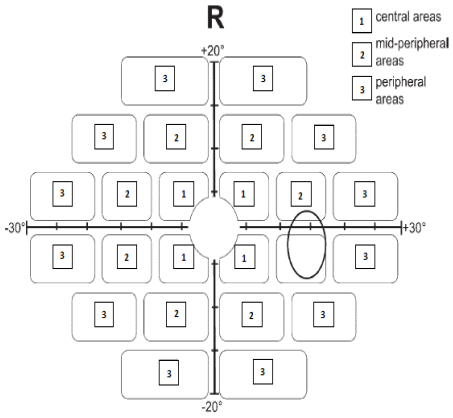

Figure 1: The visual field tested with RBP was divided into three zones: central (1), midperipheral (2) and peripheral (3).

Differences between the HFA and RBP results for the groups were evaluated using Mann-Whitney Test. Cut off value was determined between the two groups (POAG and controls) and area under the receiver operating characteristic (ROC) curves (AROC) were calculated. LE was assessed by comparing results from the three sessions with Anova test and Sidak test. TRV was evaluated by calculating differences between retest for each combination of single tests. A p value of <0.05 was taken as statistically significant.

III. Results

The controls were significantly younger than patients with preperimetric glaucoma and patients with POAG (Mann-Whitney Test, p=0.041 and p=0.026).

The HFA-MD values were significantly higher in the POAG group than in preperimetric glaucoma group and control subjects (Mann-Whitney Test, p<0.001). There was no statistically significant difference in HFA-MD values between preperimetric glaucoma group and control subjects. The average HFA-PSD values were again higher in the POAG group than in other groups (Mann-Whitney Test, p<0.001) and higher in preperimetric glaucoma eyes than in control eyes (Mann-Whitney Test, p=0.009).

The mean RBP-MHR of control, preperimetric glaucoma group, and POAG group were respectively 94.88% (SD 2.721; range 87.00-99.00); 87.42% (SD 4.56; 74.00-92.00) and 79.71% (SD 6.84; range 65.00-89.00). The differences between the groups were statistically significant (Mann-Whitney Test, p<0.001).

Cut off value for discriminating between healthy subjects and patients with preperimetric and POAG was 91.50%. Sensitivity, specificity and AUROC were respectively 0,941, 0,935 and 0.985 (p<0.001).

To evaluate LE and TRV was used data from three consecutive tests. The means and SDs of all the RBP global parameters and MMRs from the three eccentric zones (central, midperipheral and peripheral areas) were evaluated separately for controls and glaucoma patients. There were statistically significant differences in MHR, MHR-SD, MHR<90% and MMR for central and peripheral zone when comparing all three tests in the control group. There were no statistically significant differences between analyzed parameters in the POAG group except for the parameter MHR<90% (ANOVA test, p=0.018). Paired comparisons between the three tests were made with Sidak and are shown on Table 1.

Table I. Paired analysis of the parameters MHR, MHR-SD%, MHR<90% MMR from the three RBP tests (Sidak Test).

Parameter	Controls			POAG		
	Test 1 - Test 2	Test 1 - Test 3	Test 2 - Test 3	Test 1 - Test 2	Test 1 - Test 3	Test 2 - Test 3
	p	p	p	p	p	p
HR%	0,757	**<0,001**	**0,019**	0,597	0,172	0,197
MHR-SD%	0,460	**<0,001**	0,120	0,913	0,290	0,337
#MHR<90%	0,816	**0,003**	**0,013**	0,353	**0,018**	0,111
MMR CZ %	0,118	**0,040**	0,801	0,099	0,321	0,667
MMR MPZ %	0,916	0,381	0,371	0,832	0,748	0,903
MMR PZ %	0,512	**0,025**	**0,021**	0,478	0,094	0,063

The variability in the results of RBP was investigated by determining the average value, standard deviation and 95% confidence interval of the differences in retest for each combination of tests. The results are shown on Table 2 and Table 3.

Table II. Mean, standard deviation and 95% confidence interval of the differences at retest for every combination of tests conducted with RBP in the control group.

Parameter	N	Mean	SD	95%CI		Min	Max
MHR%	30	2,56	1,91	1,84	3,27	0,00	9,33
MHR-SD%	30	3,14	1,80	2,47	3,82	0,27	6,20
#MHR<90%	30	2,49	1,68	1,86	3,12	0,67	8,00
MMR CZ %	30	3,25	3,78	1,83	4,66	0,00	18,67
MMR MPZ %	30	3,43	1,98	2,69	4,17	0,67	7,17
MMR PZ %	30	3,37	2,53	2,43	4,32	0,61	9,05

Table III. Mean, standard deviation and 95% confidence interval of the differences at retest for every combination of tests conducted with RBP in the POAG group.

Parameter	N	Mean	SD	95%CI		Min	Max
MHR%	31	4,12	2,65	3,15	5,09	0,67	11,33
MHR-SD%	31	4,08	2,43	3,19	4,97	0,40	10,07
#MHR<90%	31	2,60	1,35	2,11	3,10	0,00	5,33
MMR CZ %	31	8,95	8,76	5,73	12,16	0,00	41,67
MMR MPZ %	31	5,36	4,47	3,71	7,00	0,00	21,00
MMR PZ %	31	4,17	2,79	3,14	5,19	0,00	9,78

IV. Discussion

Although SAP remains the most commonly performed method of visual field assessment in glaucoma it is relatively insensitive to early functional damage [2]. Several perimetric tests have been developed to address the fundamental aim to reveal early or low-degree neural damage. Most of these tests are function-specific, developed to target specific supgroups of retinal ganglion cells. RBP is a nonconventional perimetric test with totally different concept.

The main idea of RPB is that although the total number of ganglion cells may differ in the general population, when the retinal-cortical neural channels are intact, there are no "gaps" in the VF and thus permitting the detection of dots of opportune size, contrast, and separation [13]. A depleted neural matrix may cause "gaps" in the VF, giving rise to the detection of just one or no targets in these areas and thus a reduction in hit rates [11]. Additionally, some misses should be attributed to physiological reasons: blind spot, angioscotomata, age related losses of retino cortical neural channels, blinks, attention lapses [14, 15].

The results for mean RBP-MHR obtained by our controls (MHR 94.71%; SD 2.58%) were consistent with that reported by other authors [11, 16, 17]

The mean RBP-MHR decreases from controls to POAG group. The differences between the groups were statistically significant. The differences were statistically significant not only for controls to POAG group but also when controls were compared to preperimetric group. This means that the method is sensitive to minor damage of visual field integrity. This advantage in RBP can be attributed to various causes. First of them refers to test target parameters which include target dimensions, in space, in time, and in contrast [11].The test utilizes microdots which refers to resolution limits. Dot sizes were set to one-half of average normal minimum angles of resolution. The test uses pairs of microdots which are widely separated. This is employed to ensure that they will fall to different receptive fields. The dots were flashed in tenths of seconds with maximum contrast. Second is the different principle implemented in the test. Because of the test target parameters natural eye movements prevent repeated tests in exactly defined locations which make threshold measurements inapplicable. Instead,

the new test simply probed for the presence of vision at a large number of separate locations. The third cause is lower TRV which was reported in the literature [17, 18, 19].

Our study found that the average variability in results is moderate for MHR and MMR in the control group. The results for MHR are consistent with those published in the literature [17], and even lower for MMR.

The average value for variability of the results for MHR is significant in the group with POAG compared to the control group, and even higher for MMR especially in the central zone and in the middle periphery. So far there is no published data available in the literature for variability of the RBP results for patients with glaucoma. The high variability in the midperipheral zone can be explained by the presence of blind spot there. RBP provides no monitoring of the fixation during the test, but instead targets are occasionally projected in the blind spot to test for this. Nonmonitored eye movements and head movements probably also play a role for variability, falsely increasing sensitivity in this area.

The reason for the observed higher variability in the central area may be associated with the distraction of the attention of the patient and loss of concentration induced by interruption of the test by changing the distance for examination and the optical correction in order to investigate the central 4° of the visual field. It should also be borne in mind that test points appear smaller from a distance of 1 meter. The variability is largely associated with fatigue during the test, which in this case probably has less significance because of the short duration of the RBP test.

Paired comparison presented in Table 1 shows statistically significant LE between the second and the third test for two of the global parameters (MHR and MHR <90%) and for the peripheral zone MMR in the control group. Likely the changes in the global parameters are due to the impact of those fields with eccentric location. Otherwise LE is more pronounced when comparing the first to the last test and only in the control group. Similar study investigating the LE on results in healthy subjects did not show significant difference in the values of parameters surveyed between tests [17]. Similar results were obtained by Frise'n in a study of a group of younger volunteers [11]. He concluded that sometimes the effect of training was statistically significant but generally small.

The LE on RBP results so far has not been studied in patients with glaucoma. According to our results, there is no statistically significant LE in the group with POAG except for MHR<90% when comparing the first with the last test (Table 1). This is probably due to the fact that patients with glaucoma have more experience in performing perimetry at all.

V. Conclusion

RBP is accessible, fast and interesting test with totally different principle compared to SAP. The variability of the results is significant in the group with POAG compared to the control group especially concerning areas with midperipheral location. Probably this disadvantage can be overcome using chin rest and fixation monitoring. However RBP testing did not show significant LE in POAG group probably due to higher experience of glaucoma patients in performing perimetry. This fast and easy to perform test can successfully discriminate between healthy eye and those with early glaucoma changes and can be implemented in clinical practice especially in cases when SAP is not appropriate.

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Author M. Benova declares that she has no conflict of interest. Author I. Tanev declares that he has no conflict of interest.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

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