• Clinical Research •

Flicker defined form and RareBit measurements lack in specificity of visual pathways

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Abstract

● **AIM:** To evaluate the different visual pathways represented by the Heidelberg Engineering Perimeter flicker defined form and RareBit (magnocellular and parvocellular respectively) in different age-groups and according to the fatigue.

● **METHODS:** Totally 64 eyes of 32 healthy subjects were included in the prospective study. Each participant underwent screening—ophthalmic examination including best-corrected visual acuity, anterior and posterior segment assessment, and visual field examination with Heidelberg Edge Perimetry (HEP)-standard automated perimeter (SAP) 24-2. They were observed for 2y previously to the enrollment. This helped to define that the enrolled patients did not bear the glaucoma-developing potential. During the screening and after two years the HEP had been conducted in the standard protocol 24-2 and RareBit perimetry (RBP) in accordance with the manufacturer's description. Participants were randomly assigned to the groups: flicker defined form (FDF)-first or RBP-first. This defined from which additional visual field test the participant started. Participants were additionally subdivided to younger and older study groups. The effect of subject variables was explored with Mann-Whitney *U*-test. Testing for the presence of correlations between parameters was performed using the Spearman Rank Order Correlations and confirmed by the parametric tests. For the influence of additional factors, the Kruskal-Wallis test was performed.

● **RESULTS:** The positive correlation between mean deviation (MD) and mean hit rate (MHR) and pattern standard deviation (PSD) and standard deviation of MHR (±MHR) were found in younger study group (P=0.005, r=0.481 and P=0.0074, r=0.465), whereas in the older subgroup no correlation was observed. Additionally, the randomization protocol helped in defining the role of fatigue on the HEP-FDF results. Participant for whom the HEP-FDF was performed after RareBit had significantly worse results than those for whom the HEP-FDF was first. In the younger group, the MHR and ±MHR depend from age in that group (P<0.05, r=0.43 and r=-0.57 respectively) while no age-dependent differences were found in HEP-related parameters. On the contrary in the older group the MD and PSD varies with age (P<0.05, r=0.47 and r=-0.44 respectively) while the RBP parameters remained unchanged. The questionnaire showed that participants preferred RareBit over HEP-FDF in terms of a duration time, comfort, understanding of the test procedures, and ocular pain (P<0.05).

● **CONCLUSION:** The influence of patient's fatigue should be considered during HEP-FDF examination. An overlap hypothesis should be reevaluated after determining of other factors that affect HEP-FDF and RareBit results.

● **KEYWORDS:** perimetry; RareBit; flicker defined form
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INTRODUCTION

A Heidelberg Engineering Perimeter is one of the newer devices to diagnose and monitor progression in glaucoma. The machine was presented at the World Glaucoma Congress in Singapore in 2007. It employs flicker defined form (FDF) to detect early changes in the field of vision as well as standard automated perimeter (SAP). FDF was first described by Ramachandran in 1991. This method involves applying a high frequency stimulus that generates illusory

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
- BCVA >0.8 (Snellen charts)	- Diagnosed glaucoma, cataract or macular distortions that can disturb visual field
- UCVA >0.05 (Snellen charts)	- Visual fields defect examined by the HEP-SAP 24-2 standard test
- Refractive error with spherical equivalent below or equal to 2.5 D	- Refraction > ±2.5 DS spherical equivalent
- Cylindrical component of refractive error below or equal to 0.75 DC	- Cylindrical component of the refractive error > ±0.75 DC
- NV >0.5 (Snellen charts)	- Opacities of the optical components such as cornea, lens and vitreous that can deteriorate the visual field results
- Age >18 years old	- Fundus changes that can influence the visual field results
- Written informed consent	

BCVA: Best-corrected visual acuity; UCVA: Uncorrected visual acuity; NV: Near vision; DS: Spherical dioptres.

edges. By combining the two viewing phases, the patient has the impression of seeing the contour or edge of the stimulus. The illusory edge can be percept by patient in different visual field locations. In FDF the stimulus is presented as a ring with varying size and difficulty for the patients to distinguish the ring from the background. The patient is able to see the illusion only when the dorsal magnocellular pathway of the parasol retinal ganglion cells (approximately 10% of all ganglion cells in the retina) is stimulated. Selective contour illusion allows for early detection of functional changes. However, this method uses a flash point in medium luminance (50±2 cd/m^2), it cannot be considered using purely luminance differences between background and the stimuli. Dot elements that are randomly placed within the stimulus have always the opposite spectrum of the illuminance compared to the background dots (*e.g.* when the background dots have the luminance higher than the average, stimulus dots have it below average)[1]. Five-pronged, round stimuli are created by reversing the flickering phase of black and white dots—thereby creating the illusion of contour (areas of counterphases flickering regions of dots with high temporal frequency). It is worth to underline that flickering inside and outside of the presented stimulus occur in the same time (temporal phases are equal) but the illuminance phases are misaligned temporally. Difficulty to distinguish between stimulus and background increases with increasing variability of contrast thresholds (measured in log Michelson contrast units). Values in healthy population varies between different observers from -4.54 to 0.04 dB for mean deviation (MD) and from 3.07 to 2.0 dB for pattern standard deviation (PSD)[2-6].

RareBit perimetry (RBP) was presented by Frisén[7] in 2002. RareBit is designed to detect subtle changes in the field of vision. The technique uses spatial and temporal stimuli-microdrops to avoid simultaneous stimulation of multiple receptors in the retina. One of the features of this test is the simultaneous presentation of two widely spaced dots. This method stimulates midget retinal ganglion cells. Midget ganglion cells, the largest subpopulation of ganglion cells in retina—nearly 80%, are the most abundant of all types of coil cells that mediate, for example, image resolution. They project to the parvocellular layers of the lateral geniculate nucleus. Loss of connections in the nerve channels may indicate gaps or holes in the neuronal matrix, which may contribute to vision problems. The preliminary results of the RBP method have shown that this method is suitable for early detection of damage of cells in patients with neurological problems and glaucoma. It is considered to be fast and very cheap. Observed values of mean hit rate (MHR) in healthy population varies between studies from 78% to 100% for most studies. Only one study reported significantly lower values range of 18%-97%[8].

SUBJECTS AND METHODS

Ethical Approval This is an investigator initiated, single-center, case-control study which was conducted in a health care institution, Department of Ophthalmology, Wroclaw Medical University in cooperation with Department of Optics and Photonics, University of Science and Technology in Wroclaw. This study was registered by the number of NCT03928665 (Unique Protocol ID: ST.2012.001). The project protocol has been referred and approved by the local Bioethics Committee (approval number: 510/2012). The study was performed in compliance with the provision of the Declaration of Helsinki, as well as, International Conference on Harmonisation Good Clinical Practice guidelines and local regulations. Each participant signed informed consent in two copies and received one of them before being subjected to any medical procedure.

Patients In a prospective, case-control study 32 healthy participants (16 males and 16 females, 64 eyes) were recruited. Age of the group varied between 18 and 49y.

Only healthy subjects were recruited in this study. Participant had no ocular pathology, amblyopia, cataract, media opacity or other systemic or neurological diseases that would affect the visual field. They were observed for 2y previously to the enrolment. This helped defining that the enrolled patients did not bear the glaucoma-developing potential. Additionally, participants with refractive error exceeding 2.5 spherical diopter (DS) spherical equivalent were also excluded. Full description of inclusion and exclusion criteria can be found in Table 1.

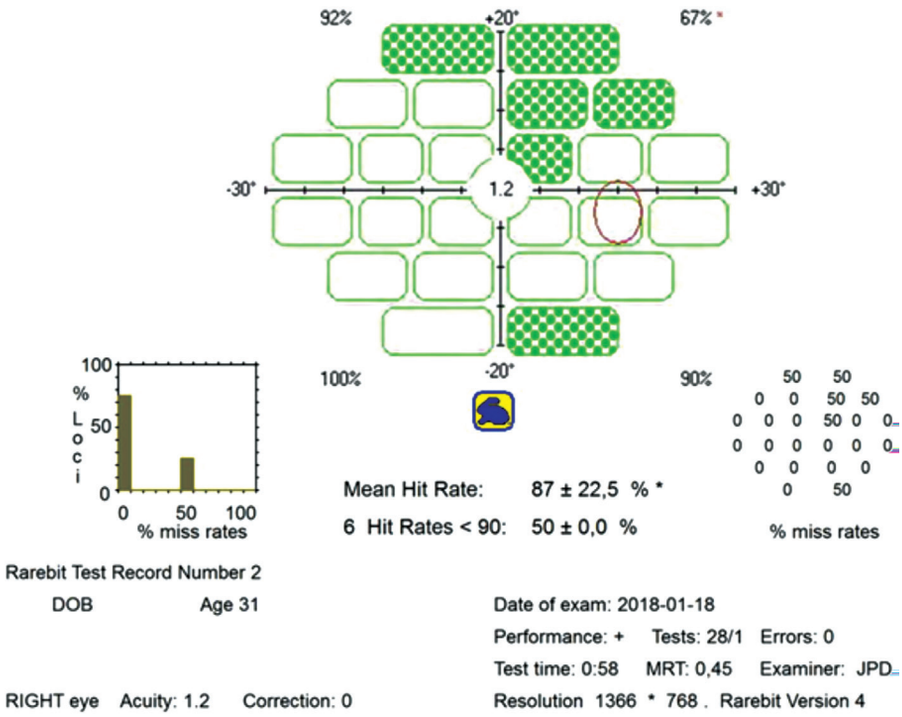

Figure 1 The example of RBP report.

Study Procedure Each patient underwent screening-ophthalmic examination including best-corrected visual acuity, anterior and posterior segment assessment, visual field examination with standard automated perimetry Heidelberg Edge Perimetry (HEP)-SAP24-2. The refractive procedures were conducted during full optometric assessment (the uncorrected-visual acuity was assessed) first monocular vision then binocular vision and balanced for accommodation demand. Then the best-corrected visual acuity was recorded using the Snellen chards. The anterior and posterior segment examination was performed by the ophthalmologist using the slit lamp (with gonioscopy and tonometry examination). After pupil dilatation the fundoscopy was performed with slit lamp and Volk lens (Super Field NC) with photograph documentation. The optic disc was assessed using the cup to disc ratio as well as disc damage likelihood scale (DDLS) and the results after 2y of observation was compared to rule out the possible glaucoma developing patients. During the screening and after 2y the HEP-FDF and RBP were recorded. The HEP-FDF has been conducted in the standard protocol 24-2 using the Adaptive Staircase Thresholding Algorithm (ASTA). The RBP version 4 for central area has been conducted using a laptop (Microsoft Windows XP, USA) with a 15″ liquid crystal display (LCD) according to the manufacturer's description[1]. The HEP-SAP 24-2, HEP-FDF 24-2 and RareBit visual field examination (Figure 1) has been performed and assessed by a trained study member (optometrist) and evaluated by an experienced ophthalmologist. Participants were randomly assigned to the groups: FDF-first or RBP-first. This defined from which additional visual field test the participant started. Participants were additionally subdivided to younger and older study groups. The randomization for the examinations and patients was obtained through free randomizer (https://www.randomizer.org/). The proposed protocol helped to distinguish the impact of fatigue on the results of the additional perimetry tests (HEP-FDF and RareBit).

Each patient has been asked to evaluate both examination procedures in terms of duration time, comfort during the test, understanding of the test procedures, comfort and ocular pain in the visual analogue scale. The questionnaire has been performed after examination procedures with distinctive indication witch perimetry patient is evaluating.

Statistical Analysis Descriptive statistics, which included means, medians, standard deviations, frequencies, lower-upper quartiles range and percentage, were used to summarize all data. The normality was assessed by Shapiro-Wilk's *W* test. The effect of subject variables (*e.g.* sex, age and refraction) on MHRs was explored with Mann-Whitney *U*-test. Testing for presence of correlations between MHR, ±MHR, MD and PSD have been performed using the Spearman rank order correlations and where the analysis of variances (Levene's test for homogeneity of variances) allowed confirmed by the parametric tests. For the influence of an additional factors the Kruskal-Wallis test was performed. For all statistical comparisons, a *P*-value of less than 0.05 was considered statistically significant. The statistical analysis was carried out in STATISTICA program.

Table 2 The normative values of the various field tests and 95%CI mean±SD (median)

Parameters	All normal subject (*n*=64)		Mature age group (*n*=32)		Young age group (*n*=32)	
	Estimated population	95%CI	Estimated population	95%CI	Estimated population	95%CI
MD (dB)	-4.45±2.7 (-3.86)	-6.75<μ<-1.19	-4.14±2.7 (-3.58)	-6.31<μ<-0.54	-4.76±2.7 (-4.24)	-6.92<μ<-1.19
PSD (dB)	2.41±1.1 (2.19)	1.50<μ<3.72	2.30±1.2 (2.07)	1.32<μ<3.92	2.53±0.9 (2.48)	1.79<μ<3.74
MHR (%)	76.81±13.8 (79.5)	71.07<μ<93.50	75.53±15.7 (80.5)	78.94<μ<96.41	78.09±11.7 (81)	68.75<μ<93.59
±MHR (%)	22.70±10.8 (22.75)	13.47<μ<35.82	21.13±8.4 (23.05)	14.38<μ<32.31	24.28±12.8 (22.75)	14.05<μ<41.23
MRT (s)	0.51±0.08 (0.5)	0.44<μ<0.60	0.51±0.09 (0.5)	0.45<μ<0.62	0.50±0.08 (0.5)	0.44<μ<0.61

The normative values for mean reaction time in each group. CI: Confidence interval; MD: Mean deviation; PSD: Pattern standard deviation; MHR: Mean hit rate; ±MHR: Probability of hit during the test; MRT: Mean reaction time; μ: Estimated population mean hit rate; SD: Standard deviation.

Table 3 Demographic data of the subjects divided into the age groups

Parameters	All normal subject (*n*=64)	Mature age group (*n*=32)	Young age group (*n*=32)
Median age (y)	31.5	39.5	22
(lower-upper quartile)	(22.0-39.5)	(35-46.5)	(21-23)
Gender (F:M)	1:1	1:1	1:1

Table 4 Correlation with age measured variables in different study populations

Parameters	All normal subject (*n*=64)	Mature age group (*n*=32)	Young age group (*n*=32)
MD (dB)	*P*=0.03, *r*=0.279	*P*=0.0064, *r*=0.471	*P*>0.05
PSD (dB)	*P*=0.0065, *r*=-0.337	*P*=0.0116, *r*=-0.441	*P*>0.05
MHR (%)	*P*>0.05	*P*>0.05	*P*=0.0136, *r*=0.432
±MHR (%)	*P*>0.05	*P*>0.05	*P*=0.0007, *r*=-0.57
MRT (s)	*P*>0.05	*P*>0.05	*P*>0.05

RESULTS

Thirty-two participants (64 eyes) were involved in our study. There were equal proportion of both sex: 16 males and 16 females. The median age was 31.5y (lower-upper quartile range: 22-39.5). The evaluated variables did not present the normal distribution. The overall means±SD (median) were -4.45±2.7 (-3.86) dB, 2.41±1.1 (2.19) dB, 76.81%±13.8% (79.5%) and 0.51±0.08 (0.5)s respectively for MD, PSD, MHR and MRT (Table 2). Estimation of the population mean was performed using the 95% confidence interval (95%CI). There was no significant effect of the gender of the subjects on the MD, PSD, MHR and MRT values. We noted a significant weak positive linear relationship between the MD and MHR values on one hand and the PSD and ±MHR on the other (*P*=0.03, *r*=0.27 and *P*=0.02, *r*=0.29 respectively). In our study there was no correlation found between age of the subjects and MHR or MRT.

In view of these findings the study results were further analysed with the division of the study population into two main groups based on age: mature and young. Table 3 presents the demographic of the two groups, while Table 2 shows overall means±SD (median) and 95% CI of MD, PSD, MHR, ±MHR and MRT.

In the subgroups based on age we found strong positive correlation between MD and MRH and PSD and ±MHR in the younger patients (*P*=0.005, *r*=0.481 and *P*=0.0074, *r*=0.465), while in older group, the correlations were not found. Additionally, in age-groups correlations with different HEP-FDF and RBP parameters were found. In younger group the MHR and ±MHR depends from age in that group (*P*<0.05, *r*=0.43 and *r*=-0.57, respectively) while no age-dependent differences were found in HEP-related parameters. On the contrary in the older group the MD and PSD varies with age (*P*<0.05, *r*=0.47 and *r*=-0.44, respectively) while the RBP parameters remained unchanged (Table 4).

During the analysis we also considered the fatigue of participants, for that purpose we used the randomization protocol to assigned each patient to different subgroup: FDF-first, RBP-first. If the HEP-FDF was conducted as the last examination procedure the results were significantly worse (*P*<0.0001 for both MD and PSD). On the other hand, no such influence on RareBit results was observed (Figure 2).

Questionnaire showed that patients prefer RBP over HEP-FDF in terms of duration time, comfort during the test, understanding of the test procedures and ocular pain (*P*<0.05 for all measured data).

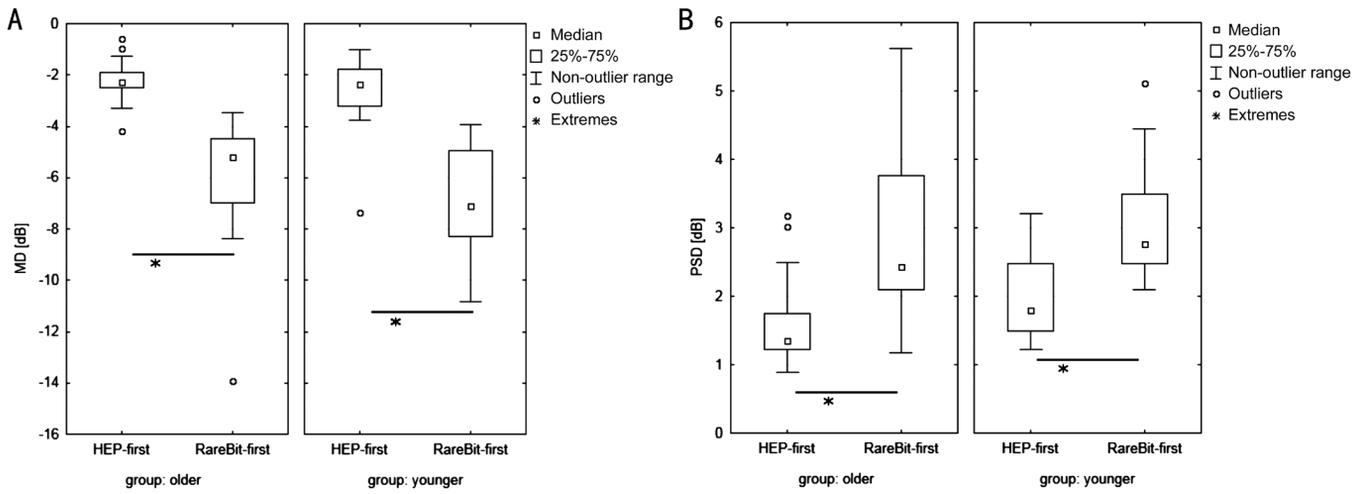

Figure 2 The influence of fatigue on the results of HEP-FDF perimetry in the younger and older group A: HEP-FDF MD (dB); B: HEP-FDF PSD (dB).

DISCUSSION

According to the best of our knowledge we present the first study that compares the HEP-FDF and RBP. Both procedures are focused on the different ganglion cells and both appear to be useful in the preperimetric glaucoma as some authors indicate. In the recent studies HEP-FDF and RBP in healthy population have been described (Table 5).

RBP is an easily achievable method, which patients find easy to understand and perform. Thanks to the Goteborg University researchers, software is now available for free use. It could be used at almost every computer without need for additional equipment or special location. Main parameter taken into consideration in RBP is MHR. According to accomplished publications it's range extends from 78% to 100% (Table 4)[7,9-13]. Only one study reported significantly lower values-range 18%-97%[8]. The comparison of the previous studies is difficult due to the different study size, ancestry, different study protocols. When comparing our results to those from literature, we used the weighted average (WA, *e.g.* $\overline{MHR}$) to rule out different sample size and possible true-healthy participants bias. In some of the presented studies there is no information about pre-enrolment observation period. Taking into account this, in previous studies normal values were 89.6%±7.62% for $\overline{MHR}$, compared to 76.81%±13.8% (95%CI: 71%-93.5%) in our study.

$$\overline{MHR} = \frac{\sum_{i=1}^{k} \dot{MHR}_i n_i}{n}$$

where:

$\overline{MHR}$ – weighted average of mean hit rate,

$\dot{MHR}_I$ – middle of the *i*-th class interval

k – number of classes,

n_i – size of the *i*-th class.

The reaction time in our study was similar to the previous ones, where it was 0.70±16s in control group[9], 0.76 (0.73-0.92)s for younger group and 0.69 (0.68-0.77)s for older group[11]. In our research the average test duration time was 289±28s which is similar to previous findings where it was 314±22s[7,9] and 268±34.1s[13]. According to Martin[11], patients preferred RBP than frequency-doubling technology perimetry (FDT) and considered it amusing and entertaining. Only the youngest participants with immature motor functions experienced difficulties with quick computer mouse double-clicking. The second group, which was not able to achieve a satisfactory result in previous studies, were advanced elderly people—not familiar with the PC mouse[12]. It stays in accordance with our observation, where young adults and middle-aged patients found RBP accessible and comprehensible.

FDF examination is much more demanding. It requires professional equipment (HEP). Patient is expected to stay focused for a longer period of time and it is hard to explain what should be seen on the test screen. It can be found difficult to perform for patients with manual disorders and those who tend to get tired easily[14-15]. Confirmation of its difficulty, might be the fact that in Reznicek *et al*'s[16] study. Totally 15% of patients suspected of glaucoma were not able to accomplish the FDF test. However, it is a widely used method. Prokosch and Eter[4] draw attention to the highest sensitivity of FDF for the detection of visual field loss corresponding with early changes in retinal nerve fiber layer thickness, followed by FDT matrix and SAP. In consequence, many authors indicate its high usefulness in diagnosing early stages of glaucoma. MD and PSD are the most important parameters. Recent studies presented values from -4.54 to 0.04 dB and from 3.07 to 2.0 dB while our research -4.45±2.7 dB and 2.41±1.1 dB, 95%CI: from -6.75 to -1.19 dB and from 1.5 to 3.72 dB for MD and PSD respectively. Interestingly, if we consider the WA, we found that normal values reported by researchers differ significantly: -0.98±1.69 dB and 2.24±0.77 dB respectively for $\overline{MD}$ and $\overline{PSD}$.

Table 5 Review of previous studies of population normative values of the HEP-FDF and RareBit central field test

Source	Examination technique	Study population	Sample size	MHR±SD (range), %	MHR, 95%CI (range), %	MD±SD/PSD±SD (range)	MD/PSD, 95%CI (range)
Frisén, 2002	RareBit	Adults	27		96±nd (88-100)		
Frisén, 2003	RareBit	Adults	15	96.6±2 (nd)			
Martin and Wanger, 2004	RareBit	Adults	54		97 (94.5-96.7) (78-100)		
Martin, 2005	RareBit	Children (6.5-12y)	21		93 (90-95) (78-100);		
		Young adults (14-20y)	30		97 (95-98) (89-100)		
Brusini *et al*, 2005	RareBit	Adults	41	88.6±4.8 (78-98)			
Salvetat *et al*, 2007	RareBit	Adults	75	91±5.7 (78-99)			
Chin *et al*, 2011	RereBit	Adults	54	86.3±14 (18-97)			
Ichhpujani *et al*, 2015	HEP-FDF	44	13			-4.54±3.95/3.07±1.37	0.70-0.95/0.27-0.60
Horn *et al*, 2014	HEP-FDF	202	60			0.04±1.8 (-3.7-5.6)	
Prokosch and Eter, 2014	HEP-FDF	91	51			-2.54±0.3	
Horn *et al*, 2015	HEP-FDF	171	60			-0.4±1.8	
Reznicek *et al*, 2015	HEP-FDF	111	46			-1.6±2.3/2.0±0.6	
Horn *et al*, 2016	HEP-FDF	122	50			0.16±1.7	

MHR: Mean hit rate; SD: Standard deviation; MD: Mean deviation; PSD: Pattern standard deviation; CI: Confidence interval; nd: Not determined.

Due to lack of common measurement units, comparison of RBP and FDF is very difficult. Similarity between results may appear because of the overlap phenomenon (non-specific stimulation of ganglion cells) or presence of some other kind of cells, which are activated in both methods. According to present knowledge retinal ganglion cells stimulated in visual field examinations are magnocellular pathway in FDF[17] and parvocellular system in RBP[18]. In our study we found positive, weak but statistically significant correlation between MD and MHR. It stands in contrast to weak negative correlation between PSD and MHR. Additionally, when the subdivision based on the age was performed, we observed that the positive correlations between MD and MHR. A PSD and ±MHR have been observed only in the younger subgroup and not in the older. It seems the correlations exists due to the changes, that appears with age and we found the weak correlation in the whole group only due to the stronger correlation in the younger group and equal subgroup sample size. These findings speak for the theory of overlap phenomenon as the retinal ganglion cells die during humans' aging process. However, taking into account the nonspecific, linear, age-dependent death of retinal ganglion cells (approximately 0.12 μm/y and 1.61 μm/decade) - the only well-defined factor, the correlation should be present in all study subgroups[19]. As this not has been shown in our study, the retinal ganglion cell loss might not be the only factor contributing to the observed changes.

In the older subjects the HEP-FDF and RBP could be considered as the selectively examining different pathways (magnocellular and parvocellular). However, this may be stated only after determining and taking into account the additional factors that could influence the examination results.

Additionally to these findings, the randomization protocol when considered into the analysis revealed an important feature of HEP-FDF results: fatigue of the participants as additional factor influencing the MD and PSD. Lamparter *et al*[20] in 2011 showed significant learning curve in the HEP-FDF performance and suggested that the rest should be offered between tests for both eyes. In our study we show that even if the rest was offered between the examination of the 1st and 2nd eye still the fatigue caused by other procedures is present. In our opinion the HEP-FDF should be performed as first examination during the patients visit. On the contrary we can use more fatigue-resistant perimetry examination such as RBP.

In our study, we found only weak positive correlation between measurements driven from HEP-FDF and RBP only in the group of the young participants. This suggest that different types of retinal ganglion cells are involved in during the examination procedures. Additionally to this we found that in different age groups there were a age-related correlations with HEP-FDF or RBP parameters (Table 4). Taking into

account that retinal ganglion cells die physiologically with age by apoptosis. Our results could be interpreted as the retinal ganglion cells that dies with age are those predominantly related with HEP-FDF stimuli. The presence of weak correlation between RBP and HEP-FDF parameters in the younger group along with age-dependent changes of MD and PSD in older group confirms this theory. In this light the magnocellular pathway will be related predominantly to the age-related changes. The process of apoptosis is highly increased among glaucoma suspected patients. Some authors indicate potentially destructive factors which may cause retinal damage by reactive oxygen species (ROS) synthesis. Most of them are proteins, *e.g.* annexin V[21] or the large group of mitogen-activated protein kinases (MAPKs)[22]. Searching for these factors is very valuable direction, which may benefit in detection of early-stages damage, before they may cause visual field loss. However, there is no data which pathway is damaged in the first place during the disease and which by the age-related changes. Our study is probably the first attempt to evaluate these different processes.

Limitations of the Presented Study Inclusion of the both eyes of each eligible participant can be a possible source of bias. Each patient underwent in one day three different visual fields tests. There is a limitation of current testing methods, *e.g.* increased measurement variability due to fatigue of participants, which could be the source of confounding results. It is also possible that there is a subject-specific factor, since in some studies the same amount of structural loss resulted in different degrees of visual field loss.

In conclusion, presence of correlations between the different examination methods such as HEP-FDF and RBP undermines their design-selectivity to one pathway, magnocellular or parvocellular. The lack of constant correlation in each age group between the MD and MHR as well as PSD and ±MHR indicates the age-related changes predominantly in the magnocellular pathway. Participants fatigue plays an important role in the HEP-FDF examination and has a significant impact on the results of visual field test.

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