INTRODUCTION:

Glaucoma is a chronic, progressive optic neuropathy marked by loss of retinal ganglion cells and their axons.

Standard Automated Perimetry (SAP) and optical coherence tomography (OCT) are two tests that assess the function and structure respectively of the retinal nerve fibres damaged in glaucoma. SAP is a psychophysical test that is patient dependent and able to detect field defects when more than 30-50 percent of ganglion cells are lost. The non-invasive Spectral Domain OCT test gives quantitative measurements of the retinal layers that are repeatable and show good agreement between the optic nerve head's anatomy and how vision works.[1]

There is a period of the disease where functional loss from RGC dysfunction occurs before cellular and axonal loss is evident on structural examination. This is the stage where Visual Electrophysiological tests can play a role.

Unlike SAP which is patient dependent, electro physiological measures of vision function are functional tests which are objective and might be more sensitive. The photopic negative response (PhNR) of the ERG and the pattern ERG (PERG) are two sensitive markers of the retinal ganglion cell dysfunction present in glaucoma.[2]

Studies have reported that PERG is very sensitive for detecting functional loss and provides a window of opportunity before apoptosis occurs. PERG can detect decreased RGC function years before structural loss in glaucoma suspects and is more sensitive than optical coherence tomography for detecting dysfunction in affected cells before damage is irreversible in ocular hypertension.[3,4]

Recently, the PhNR, a reaction triggered by RGCs receiving signals from cones, was found. Patients with primary open-angle glaucoma (POAG) have reduced PhNR amplitudes, and the degree of optic nerve damage shown by retinal nerve fibre layer (RNFL) thickness and visual field loss is connected with the drop in amplitude.[4]

There are very few research studies regarding PERG and PhNR in glaucoma suspects and their correlation with macular and peripapillary GCIPL thickness of spectral Domain OCT and MD and PSD of Standard Automated Perimeter. So, we undertook this study to understand how these two tests can be useful in glaucoma suspects.

METHODS:

This was a cross sectional interventional study conducted between July 2020 and December 2022 after receiving approval from the Institutional Ethics Committee and the study adhered to the principles of the declaration of Helsinki. All participants were recruited after informed consent.

141 individuals with 47 in each of the three groups were included in the study.
Group 1 (Glaucoma suspect) - An individual with normal visual field, intraocular pressure (IOP) under 21 mmHg, and glaucomatous optic neuropathy is considered to be a glaucoma suspect (GON) **or** Ocular Hypertensive (OHT) with IOP more than 21 mmHg, absence of GON and normal visual field **or** a person with family history of glaucoma. GON is defined as a vertical cup-to-disk ratio of at least 0.5, greater than 0.2 asymmetry, disc notching, and disc splinter haemorrhages.
Group 2 (Primary open angle glaucoma) - Patients diagnosed based on IOP > 21 mmHg, cupping >0.6 with NRR thinning and corresponding field defects and gonioscopy showing open angles with controlled IOP (<21 mmHg) by medication or laser or surgery.
Group 3 (Controls) - Subjects (25-75 years) with no glaucoma **or** family history of glaucoma study.

Myopia more than 6 D, childhood glaucoma, secondary glaucoma, primary angle closure glaucoma, poor mydriasis, other Optic neuropathies, Retinal and Neuro degenerative diseases and subjects who cannot maintain fixation are excluded from the study.

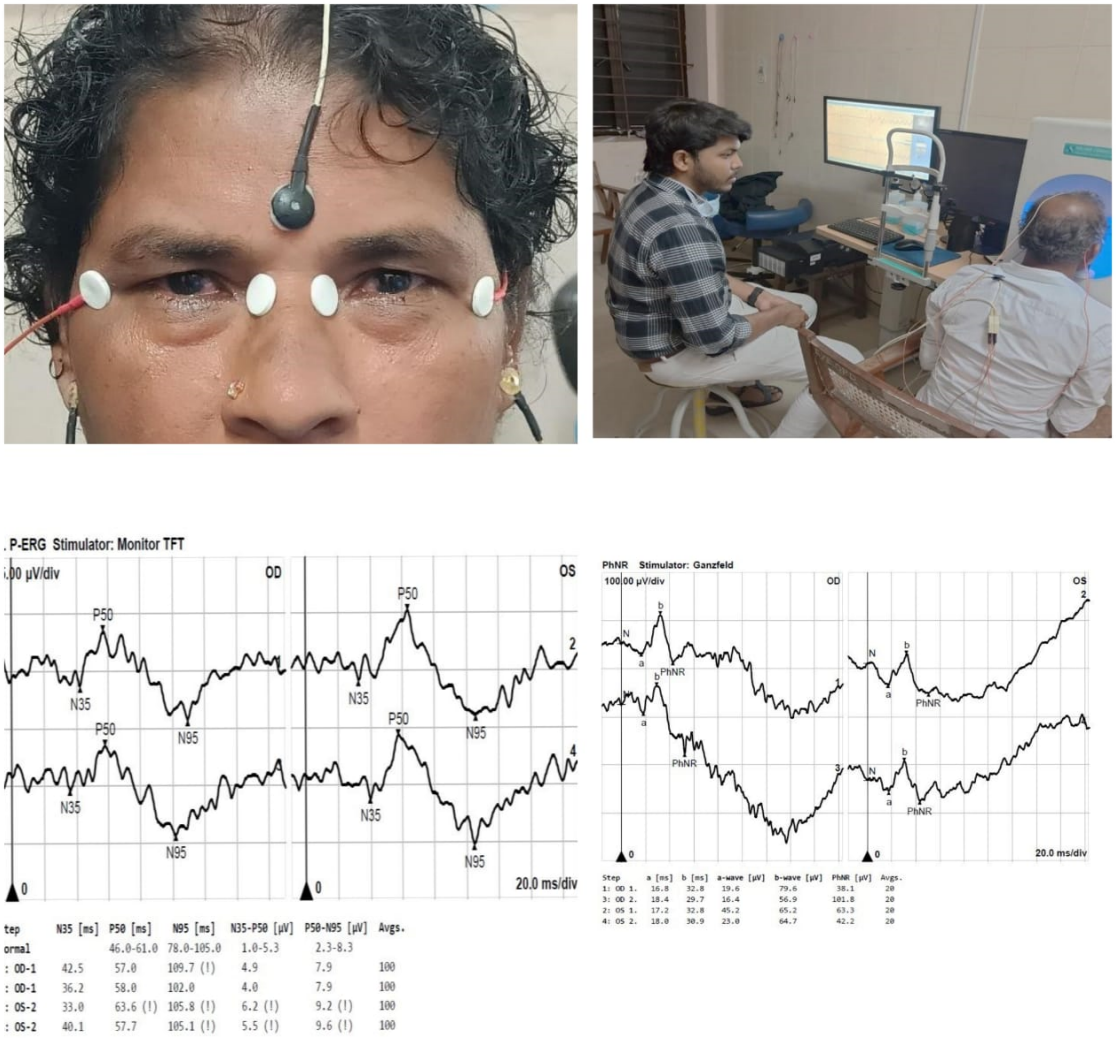
After recording basic demographic details of the patients, all patients underwent a comprehensive ophthalmological examination including anterior chamber angle measurement using a Gonio-3 mirror lens, slit-lamp bio-microscopy with 90D lens, and IOP measurement using Goldmann applanation tonometry. The retinal nerve fiber layer thickness was measured using the Cirrus HD optical coherence tomography. Visual field examination was performed using standard automated perimetry (Humphrey 860i, Carl Zeiss). PERG and PhNR were carried out in accordance with ISCEV's guidelines (International Society for Clinical Electro- physiology of Vision).

PROCEDURE:

Standard automated perimetry was used to conduct a visual field test (Humphrey 860i, Carl Zeiss Meditec Inc., Dublin,CA). The central 24-2 program and 10-2 program using the SITA (Swedish interactive thresholding algorithm) standard protocol was used for testing in all groups. Only data from reliable VF tests were taken in to account (fixation loss, false-positive and false-negative response rates of less than 20%).

Cirrus HD OCT was used to perform spectral domain OCT (OCT-500, version 10.0, Carl Zeiss Meditec Inc., Dublin,CA) to assess the peripapillary RNFL and GCIPL thickness after pupil dilation. A signal strength of more than 6 was considered for inclusion. OCT images were acquired by pRNFL scan. The optic disc's pRNFL thickness is automatically measured and analysed in a 3.46-mm-diameter circle (256 A-scan) using parameters: the average, four quadrants (superior, inferior, temporal, and nasal), the vertical cup disc ratio and rim area were noted. We considered average and quadrant thickness for our analysis. The average and minimum GCIPL thickness for both the eyes were included for our analysis.

PERG was captured binocularly using the Dawson-Trick-Litzkow electrodes (DTL) in the light-adapted state by (RETI-port/scan 21 system, Roland Consult, Germany). The two active DTL electrodes were placed in the inferior cul-de-sac whereas, the two reference electrodes are placed over the earlobes. The forehead was where the ground electrode was positioned. The patients' viewing distance from the screen was 100 cm and appropriate lens correction was given for this viewing distance. Binocular viewing of a fixation point on the screen was used while recording. The checkerboard pattern has a check size of 48min of arc that reverses 4.28 times per second. The impedance was first measured and kept less than 10kOhm. Eye movement-related data artefacts were removed. At least twice, the average of 100 responses were recorded, which takes around 30 min and these procedures are not routine OPD procedures.



tep	N35 [ms]	P50 [ms]	N95 [ms]	N35-P50 [µV]	P50-N95 [µV]	Avgs.
ormal		46.0-61.0	78.0-105.0	1.0-5.3	2.3-8.3	
: OD-1	42.5	57.0	109.7 (!)	4.9	7.9	100
: OD-1	36.2	58.0	102.0	4.0	7.9	100
: OS-2	33.0	63.6 (!)	105.8 (!)	6.2 (!)	9.2 (!)	100
: OS-2	40.1	57.7	105.1 (!)	5.5 (!)	9.6 (!)	100

Step	a [ms]	b [ms]	a-wave [µV]	b-wave [µV]	PhNR [µV]	Avgs.
1: OD 1.	16.8	32.8	19.6	79.6	38.1	20
3: OD 2.	18.4	29.7	16.4	56.9	101.8	20
2: OS 1.	17.2	32.8	45.2	65.2	63.3	20
4: OS 2.	18.0	30.9	23.0	64.7	42.2	20

Figure 1: Images of DTL Electrodes placement, PERG measurement, PERG and PhNR graphs respectively

Two negative and one positive deflection in the positions N35, P50, and N95 were present in all electrophysiological traces. We recorded the amplitude and latency of P50 and N95. The P50 amplitude was determined from the the trough of N35 to the peak of P50, whereas the N95 amplitude was determined from the peak of P50 to the trough of N95.

By using 1% tropicamide, the pupils were dilated for Multifocal ERG and PhNR, and this recording does not require visual correction. The electrode placement was same as for Pattern ERG. **Multifocal ERG** was done to detect outer retinal problems that could influence the PERG and PhNR responses.

PhNR was elicited using a Ganzfeld Q 450 Colour Dome stimulator. The impedance was first measured and kept below 10kOhm. Use of monochromatic red stimuli (625 nm) given on a 25 cd/m2 blue background was made (455 nm). A 4ms flash with a strength of 1.6 cd s/m2 was used. An average of 20 responses were obtained after the

1.25 Hz flashes were delivered. The time taken for the visual Electrophysiological tests which were not routinely done for glaucoma, ranged from 1-2 hours depending on the recordings.

Calculations were used to determine the PhNR amplitude from the baseline to the subsequent negative trough. Calculations were made to determine the ratios between the b-wave and PhNR amplitudes.

Primary outcome measures were P50, N95 amplitudes and latencies of PERG, PhNR amplitude and PhNR amplitude/b wave ratio.

Secondary outcome measures were MD and PSD of 24-2 and 10-2 VF (SAP parameters) and RNFL thickness in 4 quadrants, Average GCIPL thickness, Minimum GCIPL thickness (OCT parameters).

Sample size estimation:

Assuming prevalence of ERG changes in normal population and glaucoma suspect as 5%, 26% respectively at 95% level of confidence and 80% power, sample size was 141 (n=47 in each group) and was calculated by Epi version 3.0.1.

Statistical analysis:

Data analysis was done using Statistical Package for Social Sciences (SPSS) (version 19.0, IBM Corp., Armonk, NY) as follows: The Kolmogorov-Smirnov Test was used to verify the assumption of data normality. For non-normally distributed variables, descriptive statistics were shown as the median and interquartile range (IQR). Many groups of non-parametric data sets (p 0.5) were compared using the Mann-Whitney U test. It was carried out as a post-hoc analysis on independent samples. In nonparametric data, the relationships were statistically determined using Spearman correlation analysis. P value 0.05 was deemed statistically significant in all statistical analyses.

RESULTS:

Right eye of 141 individuals with 47 in each of the three groups were assessed. In this study, the median with IQR age of glaucoma suspects, glaucoma and controls were comparable. Males were more than the females in all three groups with a M:F ratio of 3:1 in glaucoma suspects and glaucoma group and 2.6:1 in the control group.

Demographic details and baseline characteristics of study participants are discussed in table 1.

TABLE 1:

Variable		Glaucoma suspects (n=47)	Glaucoma (n=47)	Controls (n=47)	P*
Age (Median)		55(47, 56)	56 (51, 61)	56(48, 60)	0.166
Gender	Male	35 (74.5%)	35 (74.5%)	33 (70.2%)	0.866
	Female	12 (25.5%)	12 (25.5%)	14 (29.8%)	
DM	Yes	22 (46.8%)	15 (31.9%)	20 (42.6%)	0.114
	No	22 (46.8%)	32 (68.1%)	27 (57.4%)	
HTN	Yes	24 (51.1%)	19 (40.4%)	21 (44.7%)	0.581
	No	23 (48.9%)	28 (59.6%)	26 (55.3%)	
IOP		12 (10, 14)	26 (24, 26)	14 (12, 16)	<0.001
MD (24-2) (dB)		-0.83 (-1.17, -0.43)	-9.84 (-11.27, 7.86)	-0.13 (-0.98, 0.17)	<0.001
PSD (24-2) (dB)		2.00 (1.67, 2.75)	6.87 (5.45, 8.43)	2.5 (1.75, 2.75)	<0.001
MD (10-2) (dB)		-1.12 (-1.34, -0.81)	-10.12 (-11.65, -7.38)	-0.08 (-1.10, 0.98)	<0.001
PSD (10-2) (dB)		2.25 (1.75, 2.75)	9.98 (6.84, 12.87)	2.25 (1.75, 2.75)	<0.001
Rim area (mm)		1.92 (1.45, 2.32)	0.69 (0.59, 0.76)	2.67 (1.94, 2.95)	<0.001

Vertical CD Ratio (mm)	0.65 (0.63, 0.67)	0.81 (0.79, 0.87)	0.45 (0.42, 0.49)	<0.001
Average RNFL Thickness (µm)	94 (91.5, 97)	78.25 (77.25, 80.0)	93 (90.25, 97.5)	<0.001
Superior RNFL (µm)	116 (112, 118)	94 (91, 100)	115 (109, 127)	<0.001
Inferior RNFL (µm)	120 (116, 124)	99 (95, 102)	118 (112, 128)	<0.001
Nasal RNFL (µm)	74 (69, 78)	60 (59, 62)	72 (68, 77)	<0.001
Temporal RNFL (µm)	69 (64, 72)	60 (58, 60)	61 (57, 71)	<0.001
Average GCL-IPL thickness (µm)	82 (81, 86)	70 (68, 71)	83 (80, 86)	<0.001
Minimum GCL-IPL thickness (µm)	80 (80, 82)	67 (66, 70)	82 (78, 84)	<0.001

The median with IQR for IOP in glaucoma, glaucoma suspects and controls were significantly different. The median MD (24-2) and median MD (10-2) in glaucoma were reduced when compared to glaucoma suspects and controls. The median PSD (24-2) and median PSD 10-2 in glaucoma was increased when compared to glaucoma suspects and controls. All parameters of SAP were significant with $p<0.001$. Retinal nerve fibre layer thickness and GCL IPL thickness were analysed in all the individuals. The median average RNFL and GCL-IPL thickness in glaucoma was reduced when compared to glaucoma suspects and controls respectively. The p value was significant in all the OCT parameters.

Table 2: To compare the Pattern ERG, PhNR parameters such as P50, N95 amplitudes and latencies in Glaucoma suspects, glaucoma patients and controls.

Median with IQR	Glaucoma Suspects (n=47)	Glaucoma (n=47)	Controls (n=47)	p-value	Post-hoc test***
P50 Amplitude PERG (µV)	3.02 (2.89,3.14)	2.22 (1.67,2.56)	4.86 (3.98,5.87)	<0.001*	G vs GS = <0.001 G vs C = <0.001

					GS vs C = <0.001
N95 Amplitude PERG (μV)	4.9 (4.82,5.01)	3.43 (2.99,3.87)	6.98 (6.54,7.89)	<0.001*	G vs GS = <0.001 G vs C = <0.001 GS vs C = <0.001
P50 Latency PERG (ms)	49.6 (47.3,51.7)	46.1 (43.2,48.9)	54.3 (48.7,59.1)	<0.001*	G vs GS = <0.001 G vs C = <0.001 GS vs C = <0.001
N95 Latency PERG (ms)	87.6 (78.8,92.4)	75.4 (71.1,78.7)	92.1 (87.6,100.6)	<0.001*	G vs GS = <0.001 G vs C = <0.001 GS vs C = <0.001
PhNR amplitude (μV)	24.9 (23.7, 27.9)	14.4 (13.6,15.8)	29.0 (24.7, 34.5)	<0.001*	G vs GS = <0.001 G vs C = <0.001 GS vs C = <0.001
PhNR/b wave ratio	0.18 (0.16, 0.18)	0.13 (0.11,0.15)	0.45 (0.32, 0.52)	<0.001*	G vs GS = <0.001 G vs C = <0.001 GS vs C = <0.001

G: Glaucoma, GS: Glaucoma suspects, C: Controls ,*Kruskal-Wallis H ,*** Adjusted p-value for multiple comparisons using Mann-Whitney U test.

Significant difference was noted in all three groups for pattern ERG parameters. The glaucoma suspects had significantly different values even when compared with controls. The median values of PhNR amplitudes were highest in controls followed by glaucoma suspects and glaucoma. Similarly, the median values of PhNR/b wave ratio in glaucoma were lowest followed by glaucoma suspects and controls. Significant difference noted in all three groups for pattern ERG parameters.

Table 3: Correlational analysis of PERG and PhNR parameters with OCT and SAP parameters in the Glaucoma.

r value (p-value)	P50 Amplitude PERG (µV)	N95 Amplitude PERG (µV)	P50 Latency PERG (ms)	N95 Latency PERG (ms)	PhNR amplitude (µV)	PhNR/b wave ratio
Rim area (mm)	-0.033 (0.828)	0.220 (0.137)	-0.045 (0.763)	0.056 (0.709)	-0.080 (0.593)	0.175 (0.241)
Vertical CD Ratio (mm)	0.175 (0.238)	0.156 (0.296)	0.192 (0.195)	-0.053 (0.724)	0.096 (0.523)	-0.022 (0.884)
Average RNFL Thickness (µm)	-0.191 (0.199)	-0.024 (0.874)	0.045 (0.763)	-0.070 (0.638)	-0.132 (0.378)	-0.077 (0.605)
Superior RNFL (µm)	-0.144 (0.335)	-0.182 (0.221)	-0.132 (0.376)	-0.232 (0.116)	-0.110 (0.460)	-0.157 (0.290)
Inferior RNFL (µm)	0.049 (0.745)	0.144 (0.333)	0.290* **(0.048)**	0.101 (0.500)	-0.107 (0.474)	-0.016 (0.913)
Nasal RNFL (µm)	-0.201 (0.175)	0.043 (0.776)	-0.195 (0.190)	0.055 (0.716)	-0.033 (0.826)	0.163 (0.273)
Temporal RNFL (µm)	-0.294* **(0.045)**	-0.034 (0.822)	-0.015 (0.918)	-0.136 (0.363)	0.231 (0.119)	-0.229 (0.121)
Average GCL-IPL thickness (µm)	0.051 (0.732)	0.114 (0.447)	-0.196 (0.187)	0.009 (0.951)	0.109 (0.482)	0.108 (0.471)

Minimum GCL-IPL thickness (μm)	0.024 (0.873)	-0.026 (0.864)	-0.044 (0.770)	0.160 (0.283)	0.134 (0.370)	0.104 (0.485)
Mean deviation 24-2 (dB)	-0.010 (0.947)	0.074 (0.620)	0.061 (0.686)	-0.293 (0.045)	-0.053 (0.724)	-0.028 (0.853)
Pattern standard deviation 24-2 (dB)	0.121 (0.417)	-0.007 (0.962)	-0.166 (0.266)	0.205 (0.167)	-0.005 (0.971)	0.101 (0.499)
Mean deviation 10-2 (dB)	-0.337* **(0.021)**	-0.031 (0.837)	0.104 (0.488)	-0.330* **(0.024)**	-0.027 (0.859)	-0.332* **(0.023)**
Pattern standard deviation 10-2 (dB)	0.266 (0.071)	-0.012 (0.938)	-0.135 (0.366)	0.402** **(0.005)**	0.106 (0.479)	0.268 (0.068)

****. Correlation is significant at the 0.001 level**

***. Correlation is significant at the 0.05 level**

Correlational analysis of PERG and PhNR parameters with SAP and OCT parameters were significant with strong negative correlation between MD 10-2 and P50 amplitude/N95 latency/PhNR/b wave ratio with correlation co-efficient values of -0.337, -0.330, -0.332 ($p<0.001$) respectively. Correlation was strongest between N95 latency and PSD 10-2 with correlation co-efficient value of 0.402($p<0.001$).

Table 4: Correlational analysis of PERG and PhNR parameters with OCT and SAP parameters in the Glaucoma suspects

r value (p-value)	P50 Amplitude PERG (μV)	N95 Amplitude PERG (μV)	P50 Latency PERG (ms)	N95 Latency PERG (ms)	PhNR amplitude (μV)	PhNR/b wave ratio
Rim area (mm)	-0.067 (0.653)	0.092 (0.540)	-0.083 (0.581)	-0.070 (0.642)	-0.033 (0.828)	-0.201 (0.176)
Vertical CD Ratio (mm)	0.067 (0.655)	0.112 (0.453)	0.090 (0.549)	0.162 (0.276)	0.143 (0.338)	0.069 (0.645)
AverageRNFL Thickness (μm)	-0.282 (0.055)	-0.089 (0.553)	-0.120 (0.421)	0.088 (0.557)	0.150 (0.315)	0.163 (0.274)
Superior RNFL (μm)	-0.207 (0.164)	-0.122 (0.415)	-0.067 (0.653)	0.020 (0.895)	0.065 (0.662)	0.125 (0.401)
Inferior RNFL (μm)	-0.252 (0.087)	-0.182 (0.221)	-0.039 (0.796)	0.059 (0.694)	0.097 (0.515)	0.118 (0.431)
Nasal RNFL (μm)	-0.255 (0.084)	-0.072 (0.630)	-0.124 (0.408)	0.134 (0.370)	0.079 (0.596)	0.127 (0.396)
Temporal RNFL (μm)	-0.204 (0.168)	-0.052 (0.727)	-0.217 (0.143)	0.056 (0.709)	0.221 (0.135)	0.153 (0.305)
Average GCL-IPL thickness (μm)	-0.038 (0.799)	0.108 (0.471)	0.029 (0.844)	0.087 (0.561)	0.214 (0.149)	0.097 (0.517)
Minimum GCL-IPL Thickness(μm)	-0.210 (0.158)	-0.044 (0.767)	0.121 (0.418)	0.237 (0.109)	0.140 (0.347)	0.108 (0.469)
Mean deviation 24-2(dB)	0.115 (0.442)	-0.050 (0.740)	0.030 (0.841)	0.197 (0.184)	0.106 (0.480)	0.111 (0.458)
Pattern standard deviation 24-2 (dB)	0.379** **(0.009)**	0.061 (0.682)	-0.076 (0.611)	0.093 (0.533)	0.179 (0.228)	0.091 (0.542)
Mean deviation 10-2 (dB)	0.141 (0.345)	-0.249 (0.091)	0.034 (0.822)	-0.003 (0.985)	-0.087 (0.562)	0.127 (0.393)
Pattern standard deviation 10-2(dB)	0.063 (0.675)	0.283 (0.054)	-0.117 (0.435)	-0.031 (0.834)	0.146 (0.328)	0.016 (0.914)

**. Correlation is significant at the 0.001 level
*. Correlation is significant at the 0.05 level

Correlational analysis of PERG and PhNR parameters with SAP and OCT parameters in glaucoma suspects showing strongest correlation between P50 amplitude and PSD 24-2 with correlation co-efficient value of 0.379($p<0.001$). Average RNFL and average and minimum GCL-IPL, MD 24-2, MD10-2, PSD 10-2 did not show any correlation with PERG and PhNR parameters.

Figure 2: Scatter plot of PSD 24-2 versus P50 Amplitude in glaucoma suspects

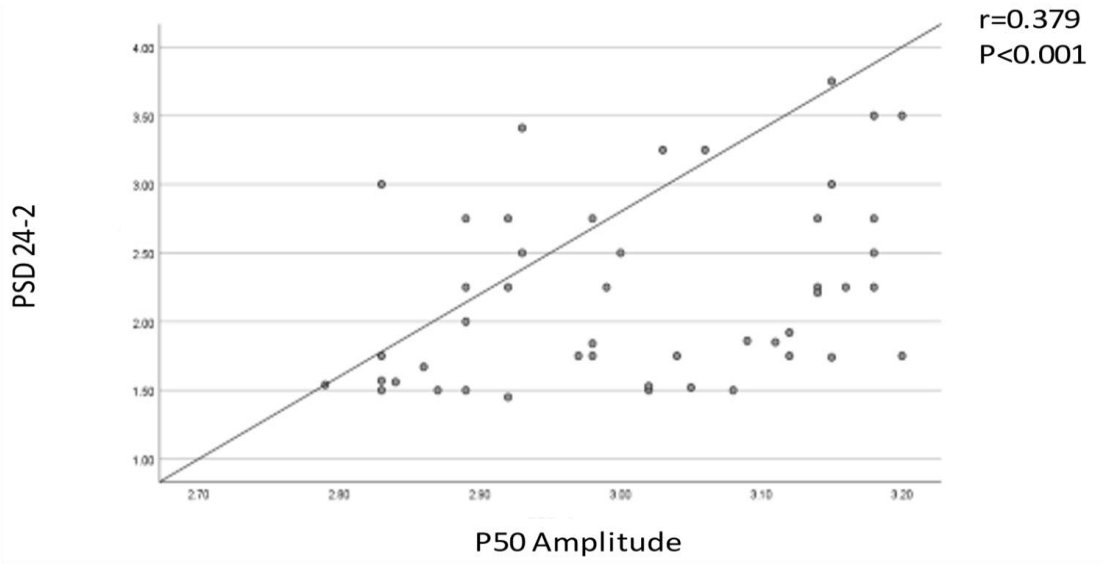


Table 5: Correlational analysis of PERG and PhNR parameters with OCT and SAP parameters in the controls.

r value (p-value)	P50 Amplitude PERG (µV)	N95 Amplitude PERG (µV)	P50 Latency PERG (ms)	N95 Latency PERG (ms)	PhNR amplitude (µV)	PhNR/b wave ratio
Rim area (mm)	0.440** **(0.002)**	-0.321* **(0.028)**	0.180 (0.225)	0.193 (0.195)	-0.186 (0.212)	0.395** **0.006**
Vertical CD Ratio (mm)	-0.195 (0.189)	0.177 (0.234)	-0.132 (0.376)	-0.001 (0.996)	0.237 (0.108)	-0.184 (0.215)
Average RNFL Thickness (µm)	-0.065 (0.665)	-0.006 (0.966)	0.091 (0.545)	-0.141 (0.343)	0.071 (0.634)	-0.400** **(0.005)**
Superior RNFL (µm)	-0.116 (0.437)	-0.184 (0.215)	0.006 (0.969)	-0.139 (0.353)	0.136 (0.362)	-0.175 (0.239)

Inferior RNFL (µm)	0.021 (0.888)	0.028 (0.852)	-0.002 (0.991)	-0.118 (0.429)	0.110 (0.463)	0.001 (0.995)
Nasal RNFL (µm)	-0.138 (0.356)	-0.001 (0.993)	0.051 (0.732)	0.153 (0.304)	-0.090 (0.546)	-0.060 (0.688)
Temporal RNFL (µm)	0.184 (0.216)	0.062 (0.678)	0.146 (0.328)	-0.037 (0.807)	-0.114 (0.444)	-0.226 (0.126)
Average GCL-IPL thickness (µm)	-0.083 (0.578)	-0.107 (0.473)	0.137 (0.358)	-0.158 (0.287)	-0.179 (0.229)	-0.077 (0.606)
Minimum GCL-IPL thickness (µm)	-0.128 (0.392)	-0.171 (0.251)	0.164 (0.272)	-0.238 (0.107)	-0.216 (0.145)	-0.064 (0.671)
Mean deviation 24-2 (dB)	-0.268 (0.069)	-0.049 (0.743)	0.021 (0.889)	0.130 (0.385)	-0.070 (0.638)	-0.171 (0.251)
Pattern standard deviation 24-2 (dB)	-0.066 (0.659)	0.071 (0.637)	0.017 (0.909)	-0.125 (0.402)	0.164 (0.271)	-0.309* **(0.034)**
Mean deviation 10-2 (dB)	-0.139 (0.353)	-0.167 (0.655)	-0.042 (0.780)	0.246 (0.096)	-0.165 (0.268)	0.040 (0.789)
Pattern standard deviation 10-2 (dB)	-0.154 (0.301)	0.289 (0.048)	-0.058 (0.697)	-0.128 (0.390)	0.241 (0.103)	-0.385** **(0.008)**

**. Correlation is significant at the 0.001 level

*. Correlation is significant at the 0.05 level

Correlational analysis of PERG and PhNR parameters with SAP and OCT parameters in controls showing strong negative correlation between PhNR/b wave ratio and average RNFL/PSD 24-2/PSD 10-2 with correlation co-efficient values of -0.400($p<0.001$), 0.309($p<0.05$), -0.385 ($p<0.001$) respectively. Average and minimum GCL-IPL thickness and MD24-2 and MD 10-2 did not show any correlation with PERG and PhNR parameters.

Table 6: Correlational analysis of PERG with PhNR parameters in Glaucoma, Glaucoma suspects and Controls.

	r value (p-value)	P50 Amplitude PERG (μV)	N95 Amplitude PERG (μV)	P50 Latency PERG (ms)	N95 Latency PERG (ms)
Glaucoma	PhNR amplitude (μV)	0.157 (0.293)	-0.026 (0.864)	-0.011 (0.942)	0.320* **(0.028)**
	PhNR/b wave ratio	0.143 (0.339)	0.070 (0.641)	-0.078 (0.604)	-0.298* **(0.042)**
Glaucoma suspects	PhNR amplitude (μV)	0.034 (0.822)	0.247 (0.095)	-0.108 (0.469)	0.793** **(0.001)**
	PhNR/b wave ratio	0.113 (0.448)	0.186 (0.211)	0.074 (0.621)	0.676** **(0.001)**
Controls	PhNR amplitude (μV)	-0.018 (0.904)	0.436** **(0.002)**	-0.368* **(0.011)**	-0.129 (0.389)
	PhNR/b wave ratio	0.361* **(0.013)**	-0.266 (0.070)	-0.081 (0.587)	0.167 (0.262)

Correlation analysis among PERG with PhNR parameters were done among each group. Strong positive correlation was seen in glaucoma suspects among PhNR amplitude, PhNR/b wave ratio with N95 latency with $p<0.001$.

Figure 3: Scatter plot of PhNR Amplitude versus N95 Latency in glaucoma suspects

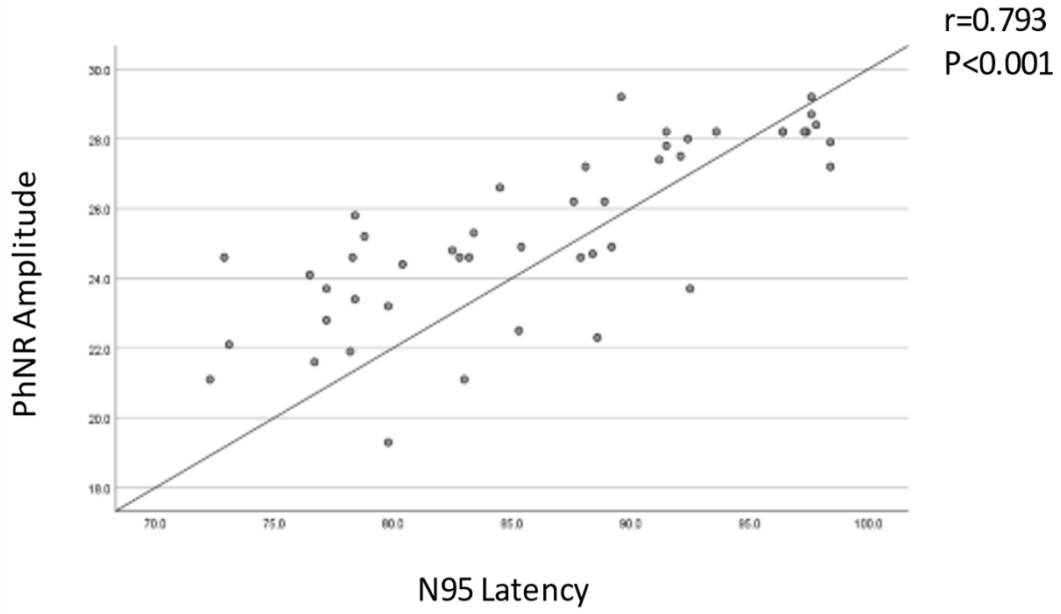


Figure 4: Scatter plot of PhNR/b wave ratio versus N95 Latency in glaucoma suspects

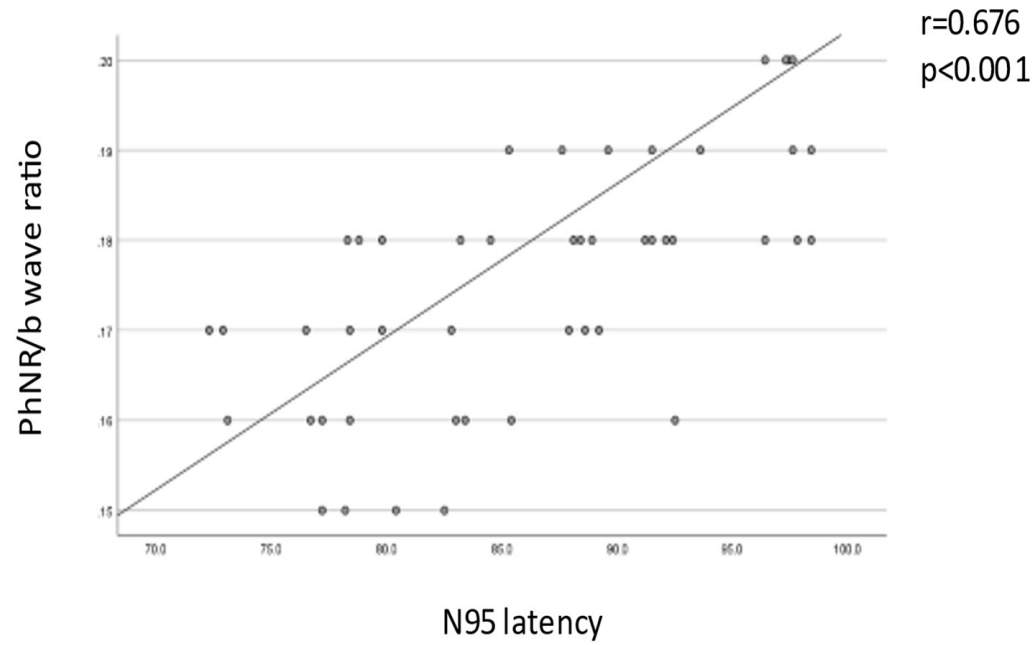


Table 7: Correlational analysis of PERG with PhNR parameters in the 3 groups.

r value (p-value)	P50 Amplitude PERG (μV)	N95 Amplitude PERG (μV)	P50 Latency PERG (ms)	N95 Latency PERG (ms)
PhNR Amplitude (μV)	0.735** (<0.001)	0.820** (<0.001)	0.388** (<0.001)	0.732** (<0.001)
PhNR/b wave ratio	0.860** (<0.001)	0.850** (<0.001)	0.534** (<0.001)	0.782** (<0.001)

****. Correlation is significant at the p<0.001 level.**
***. Correlation is significant at the p<0.05 level.**

Correlation analysis among PERG with PhNR parameters were done in all groups. Strong positive correlation was seen among all parameters of PERG and PhNR with p<0.001.

DISCUSSION:

The main purpose of this study was to find out whether the parameters of PERG and PhNR are affected in a glaucoma suspect as in established glaucoma and to find out if there was correlation with SAP and OCT parameters.

The key findings of our paper was that the amplitudes and latencies of PERG and PhNR do decrease in glaucoma suspects as compared to controls. Correlation analysis with SAP, OCT and ERG parameters was not significant in glaucoma suspects implying that electrophysiological changes precede RNFL and field changes.
In our cross-sectional study, the median age in all the three groups was similar. Although age is a key confounding factor that can alter the amplitude of PERG and RNFL thickness, there was no statistically significant age difference between any of the groups in this investigation, so the data could be compared. The gender distribution showed no significant difference.

P50 and N95 amplitude had a smaller amplitude among glaucoma suspects as in established glaucoma patients when compared to normal subjects. These results are consistent with other studies such as Ganekal et al[13], E O'Donaghue et al[33], Neoh et al[34], where PERG amplitudes are decreased. The PERG latencies (P50 and N95) were also decreased in glaucoma suspect patients in our study. Karaca et al[29] and Ganekal et al[13] found that PERG P50 (ms) and N95 latencies (ms) decreased in glaucoma suspects as compared to glaucoma and was significant ($p = 0.0012$).

Glaucoma suspects and glaucoma patients had lower PhNR characteristics (amplitude) 24.9 µV (23.7, 27.9), 14.4 µV (13.6,15.8) respectively, when compared to normal subjects 29.0 µV (24.7, 34.5). Results of our study are consistent with other studies such as Machida et al[31], Shen X et al[26], Awwad et al[27] showed decreased PhNR amplitudes in study groups compared to normal subjects.

We also performed multifocal ERG to detect outer retinal defects as it may affect the PERG and PhNR responses. As some of our subjects were diabetics those were excluded.

We found that correlation between PERG, PhNR and SAP, were not significant in glaucoma suspects as compared to established glaucoma patients, As the correlation was seen only between P50 amplitude of PERG and PSD 24-2 of SAP in glaucoma suspects **($r=0.379$, $p<0.001$)**. Results of this study suggests that PERG recognise the RGC damage earlier than SAP parameters. Sehi et al[24] evaluated that in glaucoma suspects PERGLA amplitude did not show any correlation with SAP parameters [MD r=0.21, p=0.22], [PSD r= -0.15, p=0.38]. Ventura et al[36] found that in glaucoma suspects, the PERG amplitude was weakly correlated with SAP (r=0.225, p= 0.04). The average SAP MDs (GS: -0.58 dB) and PERG deviations in glaucoma suspects (-1.13dB) were of the same magnitude. Garway-Heath et al[43] found that in glaucoma suspects, there was a small but significant correlation between PERG amplitude and SAP.

Also, in our study when we compared the PERG, PhNR parameters with SAP among all three groups (n=141), all parameters among PERG with SAP have shown strong positive correlations with $p<0.001$. As a result of the larger sample size used for the comparison of the three groups, the parameters are now more trustworthy than the individual groups.

In our study no significant correlation was noted among PERG, PhNR parameters and SD OCT parameters in glaucoma suspects. This suggests that even though PERG parameters were reduced in glaucoma suspects, no structural changes were seen or detected by SD-OCT which makes PERG an important diagnostic tool in recognising early changes (RGC damage).

Also, in our study when we compared the PERG, PhNR parameters with RNFL and GCIPL among all three groups(n=141), negative correlations were noted among vertical CD ratio and PERG, PhNR parameters. Whereas other parameters among PERG, PhNR with RNFL and GCIPL have shown strong positive correlations with $p<0.001$. This is due to increase in sample size when comparison was done among all three groups making the parameters more reliable than individual groups. Results of this study are consistent with Falsini et al[23] demonstrated that PERG amplitude did not substantially associated with RNFL thickness.

We looked in to the correlation between PERG and PhNR parameters in glaucoma suspects. Both glaucoma suspects and glaucoma patients' PhNR amplitude and PhNR/b wave amplitude ratio were correlated with N95 latency ($r=0.793$, $p<0.01$ and $r= 0.676$, $p<0.01$). Results are consistent with Preiser et al[41] found that highest correlation ($r=0.3$) was found between PERG and PhNR in glaucoma suspects. Drasdo et al[45] found moderate correlation among PhNR and PERG parameters ($r = 0.50$, $P = 0.032$) in glaucoma suspects.

We also investigated the correlation between PERG and PhNR parameters in all three groups. All the parameters of PERG and PhNR were significantly correlated with each other with $p<0.001$. This is because the sample size increased when comparisons were made across all three groups, making the parameters more reliable than within-group comparisons.

The strength of our study was it was adequately powered with equal numbers in all the three groups. The ERG wave patterns were compared to the two most important routinely performed diagnostic tests namely perimetry and OCT. A limitation could be that the glaucoma suspects were not followed up to look for those who converted to proper glaucoma.

Future directions should look at doing a longitudinal cohort study to demonstrate the ability of PERG and PhNR to track the development of glaucoma over time and evaluate its dependability in producing consistent and accurate data at different times of recording.

CONCLUSION:

Pattern ERG and PhNR showed significant changes in glaucoma suspects as compared to established glaucoma and controls. P50 Amplitude and PhNR amplitude showed strong correlation in glaucoma suspects. By comparing OCT and SAP parameters across all three groups, all parameters apart from the vertical CD ratio showed a substantial positive connection with PERG and PhNR. No correlation was seen for SAP and OCT parameters with ERG changes among glaucoma suspects implying that electrophysiological changes precede RNFL and field changes. Thus, PERG and PhNR might be useful as an adjuvant in the early diagnosis and management of glaucoma suspects especially those with high risk.

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