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Expression of poly(ADP-ribose) polymerase-1 gene and optical coherence tomography angiographic parameters among patients with multiple sclerosis

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ABSTRACT

Back ground/aims To analyse different parameters of the macula, disc and their vascular affection using optical coherence tomography (OCT) and angiography (OCT-A) in patients with multiple sclerosis (MS) correlating these changes to PARP-1 gene expression in blood.

Methods This cross-sectional study included 80 eyes of the clinically diagnosed relapsing-remitting phenotype of MS. The study included three groups; group (A) included 40 eyes of 20 patients with MS with a history of optic neuritis (MS+ON), group (B) included 40 eyes of 20 patients with MS without a history of ON (MS-ON) and group (C) (the control group) consisted of 40 eyes of 20 matched participants not suffering from any ocular or systemic disease. OCT and OCT-A, RTVue (Optovue, Fermont, California, USA) were done for all eyes for evaluating the macular and disc changes. Qualitative real-time PCR for estimation of PARP1 gene expression level was performed for all patients.

Results PARP-1 gene expression level showed a significant difference in comparing the three groups, with the highest level being for the (ON+) group ($p<0.0009$). Significant negative correlations were found between PARP-1 gene expression level and central macular thickness, total macular volume and full foveal vessel density thickness. ROC curve constructed by plotting the area under the receiver operating characteristic curve value was (0.9) for PARP-1 gene expression level.

Conclusions PARP-1 may play an important role in the development of the ON cascade in patients with MS and may be a biomarker for diagnosing and a potential molecular target of ON in MS patients' therapy. In addition to the OCT and OCT-angio changes that could be detected retrospectively, PARP-1 gene expression level could be considered a prospective detector to complete the full-blown picture of MS (ON+) early and prevent blindness.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The most common ocular presentation of multiple sclerosis (MS) is optic neuritis (ON), representing the first clinical manifestation in about 25% of cases.

WHAT THIS STUDY ADDS

⇒ PARP-1 may be a biomarker for diagnosing and a potential molecular target of ON in MS patients' therapy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Qualitative real-time PCR for estimation of PARP1 gene expression level.

INTRODUCTION

Multiple sclerosis (MS) is a disabling disease targeting the central nervous system (CNS) via an autoimmune and inflammatory route. Plaque formation is followed by the destruction of the myelin sheath resulting in axonal degeneration.[1 2]

The most common ocular presentation of MS is optic neuritis (ON), representing the first clinical manifestation in about 25% of cases.[3 4] It is confirmed that the visual system is affected through the post-mortem specimens (in the form of axonal loss and degeneration of the optic nerve) in about 94 to 99% of patients with MS whether they had previous ON or not.[5 6]

Visual pathway in patients with MS represents a directly visible common affected partner of the CNS. In order to objectively quantify and assess the visual pathway, Spectral-domain optical coherence tomography (SD-OCT) is a safe, accurate, non-invasive technique that provides micrometre axial resolution in cross-sectional retinal imaging for reliable quantification of the ganglion cell axonal layer thickness at the level of the peripapillary retinal nerve fibre layer (RNFL) and the macular region.[7 8]

OCT angiography (OCT-A) is a new, non-invasive imaging technique that produces an angiographic image in a matter of seconds

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without using dyes by using motion contrast imaging of high-resolution volumetric blood flow. The en face images of OCT angiograms can be visualised in definite layers starting from the internal limiting membrane down to the choroid, in addition to the visualisation of individual vascular plexus and segmentation of the inner retina, outer retina and choriocapillaris slabs.[9] Vascular plexus affection of the superficial and deep retinal vessels associated with ON can be the contributor to the neuronal or degenerative dysfunction in patients with MS.[10]

Poly (ADP-ribose) polymerase 1 (PARP-1) is one of the well-characterised and most popular members of the PARP nuclear enzyme superfamily, which transfers ADP-ribose units from nicotinamide adenine dinucleotide to a broad panel of acceptor proteins, such as histones and transcription factors. PARP-1 participation was identified in various cellular processes, including DNA repair, proliferation, death signalling of the cells, transcriptional regulation and inflammation. Previous studies on the experimental autoimmune encephalomyelitis model of MS pointed to the importance of PARP-1 in MS pathogenesis, suggesting that the development of PARP-1 inhibitors is a promising approach for MS treatment modulations.[11]

The study aims to objectively analyse different parameters of the macula and disc using OCT in addition to their vascular affection using OCT-A in patients with MS (with or without ON) vs the normal control group and correlate these changes to PARP-1 gene expression in blood of patients with MS. It may help predict or even prevent the development of ON and subsequent visual disabilities.

MATERIAL AND METHODS

This cross-sectional study included 80 eyes of the clinically diagnosed relapsing-remitting phenotype of MS. The patients were recruited from neurology outpatient and inpatient clinics from May 2021 to December 2021, Sohag University Hospitals.

The study included three groups; group (A) (MS+ON) had 40 eyes of 20 patients with MS with a history of ON, group (B) (MS-ON) had 40 eyes of 20 patients with MS without a history of ON,and group (C) (control group) consisted of 40 eyes of 20 matched participants not suffering from any ocular or systemic disease.

The study included patients with MS aged 18 years or more diagnosed according to McDonald's criteria 2017.[7] In groups (A and B), we included only patients at least 3 months after the resolution of the attack. All patients with MS (groups A and B) were on the same line of neurological management.

Patients with media opacity as corneal opacity or dense cataract that interferes with the signal strength of images, patients with any other retinal pathology, such as diabetic retinopathy, retinal degeneration and retinal dystrophies, and those with any other causes of optic neuropathy, including glaucoma, ischaemic and compressive optic neuropathy were excluded. Also, patients with other demyelinating diseases, for example, acute disseminating encephalomyelitis or neuromyelitis optica, were excluded. Patients with acute attacks of ON were excluded, as optic nerve head (ONH) edema would prevent the accurate measurement of RNFL. Eyes with high myopia (more than −6 SD) were rolled out.

The neurological examination was done to determine the type and the severity of MS by using the EDSS Score. The patients underwent a full ophthalmic examination, for example, intraocular pressure assessment (IOP) using the gold-standard applanation tonometry, slit-lamp examination of the anterior segment and retinal assessment by slit-lamp biomicroscopy using a+78 D lens, then OCT and OCT-A, RTVue (Optovue, Fermont, California, USA) were performed for all patients.

Table 1 Sociodemographic and clinical data among the studied group, n=120

Parameter	MS (ON+) N=40		MS (ON−) N=40		Control N=40		*P value
	No	%	No	%	No	%	
Sex							
Females	36	90.0	20	50.0	40	100.0	<0.001
Males	4	10.0	20	50.0	0	0.0	
Side							
OD	20	50.0	20	50.0	20	50.0	1
OS	20	50.0	20	50.0	20	50.0	
Age (years)							
Mean+SD	38±8		34±6		36±5		0.018

*P value was calculated by χ^2 test or one-way ANOVA test wherever suitable.
ANOVA, analysis of variance; MS, multiple sclerosis; ON, optic neuritis.

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The technique of OCT of the optic disc

A circular profile of 3.4 mm centred on the optic disc manually adjusted to the optic disc margins was taken to assess the peripapillary RNFL thickness. Mean peripapillary RNFL thickness and four quadrants thickness were evaluated. OCT of the macular ganglion cell complex (GCC), including the average and 3 mm circular area, was evaluated in the study. OCT-A: of ONH was performed using a 4.5×4.5 mm scan centred on ONH, and vessel density in the four quadrants was assessed. OCT-A of the macula 6×6 mm scan was centred on the fovea in four levels to determine vascularity at different levels. The full thickness vascular density (internal limiting membrane layer (ILM)-retinal pigment epithelium layer (RPE)) and the superficial vessel density (ILM-IPL) were determined.

Colour code was used in density map images in which the hot colours represented a more flow; hot colours (red and orange) represented functional perfusion areas, while cold colour (blue) represented low perfusion. Total macular volume (TMV), central macular thickness (CMT) and para and perifoveal sectors thickness were evaluated using a 5×5 mm scan centred on the fovea.[12]

Table 2 OCT and OCT-angio parameters

	MS (ON+) N=40			MS (ON–) N=40			Control N=40			
Parameter	Mean	±	SD	Mean	±	SD	Mean	±	SD	P value*
CMT	225	±	18	234	±	21	240	±	18	0.02
TMV	6.53	±	0.39	6.69	±	0.18	6.97	±	0.22	<0.0002
SFVD	15	+	6	24	+	2	19	+	7	<0.004
SS Para-FVD	53	+	4	50	+	4	52	+	3	0.04
SS peri-FVD	49	±	4	49	±	2	51	±	3	0.002
SN para-F VD	51	±	5	52	±	3	49	±	5	0.01
F FVD	227	±	19	239	±	14	245	±	18	0.001
FS Para-FVD	299	±	19	305	±	10	317	±	30	0.005
FS Peri-FVD	262	±	15	273	±	16	279	±	8	<0.0003
FI Para-FVD	296	±	17	298	±	5	320	±	16	<0.0009
FI Peri-FVD	252	±	12	261	±	18	271	±	12	<0.002
FN Para-FVD	302	±	35	298	±	5	312	±	17	0.004
FN Peri-FVD	266	±	22	279	±	18	282	±	15	0.006
FT Para-FVD	290	±	17	289	±	4	309	±	20	<0.001
FT Peri-FVD	250	±	15	258	±	14	272	±	23	<0.009
SRNFL	115	±	15	115	±	17	123	±	9	0.02
IRNFL	111	±	16	111	±	14	127	±	17	<0.002
NRNFL	67	±	9	70	±	12	77	±	11	0.004
TRNFL	62	±	12	56	±	8	73	±	9	<0.0007
Average GCC	85	±	9	85	±	10	97	±	6	<0.002
SGCC	85	±	9	86	±	10	111	±	67	0.02
IGCC	86	±	9	84	±	10	97	±	7	<0.002
SDVD	51	±	5	49	±	2	52	±	5	0.001
IDVD	50	±	8	48	±	4	51	±	5	0.03
NDVD	52	±	6	48	±	4	52	±	6	0.003
TDVD	50	±	5	48	±	4	51	±	5	0.05
BCVA	0.30	±	0.14	0.60	±	0.14	0.89	±	0.12	<0.007

*P value was calculated by one-way ANOVA test.
ANOVA, analysis of variance; Average GCC, average ganglion cell complex thickness; BCVA, best-corrected visual acuity; CMT, central macular thickness; FFVD, full foveal vessel density; FI Para-FVD, full inferior para-FVD; FI-Peri FVD, full inferior peri-FVD; FN Para-FVD, full nasal para FVD; FN Peri-FVD, full nasal peri-FVD; FS Para-FVD, full superior para-FVD; FS Peri-FVD, full superior peri-FVD; FT Para-FVD, full temporal para-FVD; FT Peri-FVD, full temporal peri-FVD; IDVD, inferior disc vessel density; IGCC, inferior GCC thickness; IRNFL, inferior peripapillary retinal nerve fibre layer thickness; MS, multiple sclerosis; NDVD, nasal disc vessel density; NRNFL, nasal peripapillary RNFL thickness; ON, optic neuritis; SDVD, superior disc vessel density; SFVD, superficial FVD; SGCC, superior GCC thickness; SI Para-FVD, superficial inferior para-FVD; SI Peri-FVD, superficial inferior peri-FVD; SN Para-FVD, superficial nasal para-FVD; SRNFL, superior peripapillary RNFL thickness; SS Para-FVD, superficial superior para-FVD; SS Peri-FVD, superficial superior peri-FVD; TDVD, temporal disc vessel density; TMV, total macular volume; TRNFL, temporal peripapillary RNFL thickness.

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Cell isolation

The authors isolated mononuclear cells from the peripheral blood collected on EDTA anticoagulant by density gradient centrifugation (Lymphoprep; Axon Lab, Switzerland) Phosphate-buffered saline (PBS) reagent (Biomed, Poland).

The technique of qualitative real-time PCR for the estimation of PARP1 gene expression level.

RNA isolation

RNA was isolated from the samples obtained from peripheral blood mononuclear cells. Isolation of the total cellular mRNA was carried out using GeneJET Whole Blood RNA Purification Mini Kit (Thermo scientific, USA, cat: K0761). Moreover, evaluation of RNA purity (A260/280 ratio of 2.0–2.1) and quantity was done by using the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific).

Real-time quantitative PCR

Commercially available RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA cat: K1622) according to the manufacturer's protocol was used for total RNA was reverse-transcribtion. cDNA was employed as a template for (RT-PCR). Real-time PCR on StepOnePlus (Applied Biosystems) using commercially available TaqMan probes (Applied Biosystems, USA) for endogenous control gene: PUM1 (Hs 00472881_m18); for PARP1 gene: Hs00242302 was used for performing the analysis of PARP-1 gene expression.

The qPCR reaction was carried out in 96-well-optical plates, in the volume 0 f 25 µL/well, consisting of 10.25 µL RNAz- and DNAz-free ultrapure water, 1.25 µL gene-specific probe, 12.5 µL TaqMan Gene Expression Master Mix ((Inventoried_FAM-MGB) and 1 µL cDNA synthesised in the reverse transcription reaction. The qPCR reaction, after the initial 10 min denaturation at 95°C, was carried out according to the following scheme—40 cycles: 15 s at 95°C and 60 s at 60°C.

Public involvement

Members of the public were involved in different stages of the study including design and conduct. We received input from public continuing online education and implemented them in our study design. We intend to disseminate the main results to participants of the study and will seek public involvement in the development of a suitable method of dissemination.

Statistical analysis

Data were analysed using the SPSS computer program V.25.0. Quantitative data were expressed as means±SD, median and range. Qualitative data were expressed as numbers and percentages. A χ^2 test or one-way analysis of

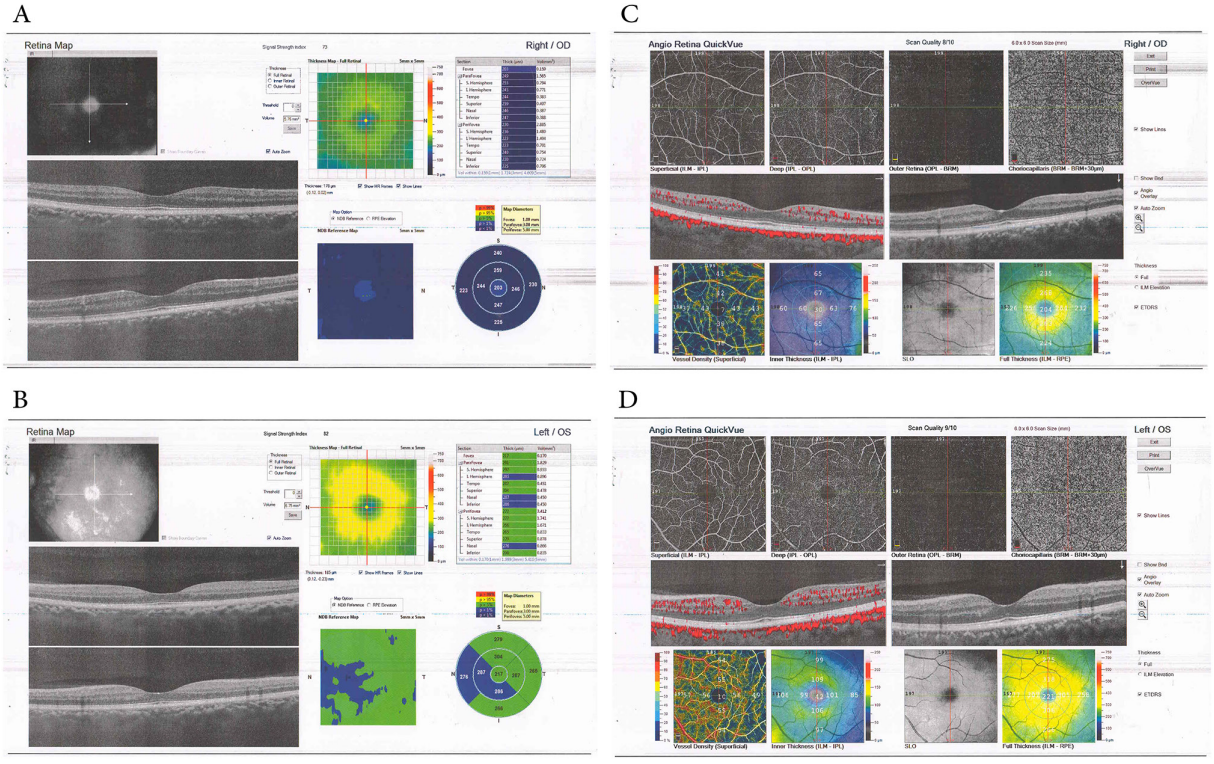

Figure 1 3D OCT macula and OCT-angiography of the macula. (A) Rt.OCT macula of patient with MS (ON+) with marked thinning of central macular thickness and total macular volume. (B) Lt.OCT macula of patient with MS (ON–) with normal central macular thickness and decreased total macular volume. (C) Rt.macula of patient with MS (ON+) showing decreased vessel density in the superficial and full thickness in the foveal, perifoveal and parafoveal sectors. (D) Lt.macula of patient with MS (ON–) with decreased vessel density in the superficial layers and full thickness of the foveal, perifoveal and parafoveal sectors. MS, multiple sclerosis; OCT, coherence tomography angiography; ON, optic neuritis.

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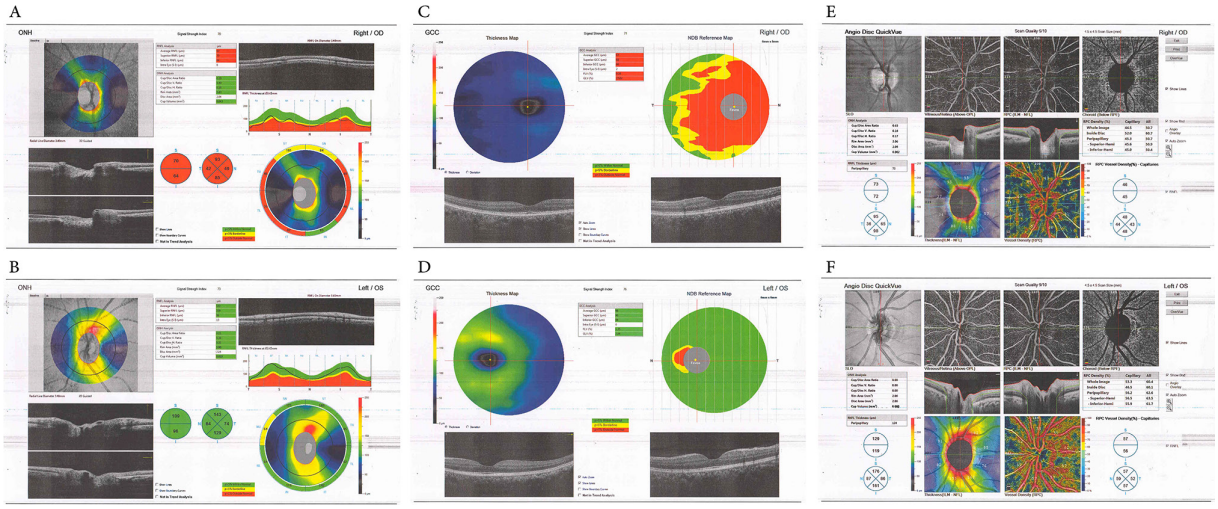

Figure 2 OCT of the optic nerve head, OCT macula-V of ganglion cell complex (GCC) and OCT-angio of the optic nerve head. (A) Rt. disc of patient with MS (ON+) showing marked thinning of the whole sectors of the peripapillary retinal nerve fibre layer. (B) Lt. disc of patient with MS (ON–) showing within the normal thickness of the peripapillary retinal nerve fibre layer. (C) Rt. GCC at the macula of patient with MS (ON+) showing marked thinning of the average, superior and inferior GCC. (D) Lt.GCC at the macula of patient with MS (ON–) showing within the normal thickness of the average, superior and inferior GCC. (E) Rt. Disc of patient with MS (ON+) showing decreased flow (highlighted in blue) with decreased vessel density. (F) Lt. Disc of patient with MS (ON–) showing decreased flow (highlighted in blue) and within normal vessel density. MS, multiple sclerosis; OCT, coherence tomography angiography; ON, optic neuritis.

variance was used to compare the results of the different groups. A 5% level was chosen as a level of significance in all statistical tests used in the study. Post hoc analysis (TUKEY) test was used for further comparison between groups. ROC curve constructed by plotting the area under the receiver operating characteristic curve (AUC).

RESULTS

The study included 120 eyes of 60 subjects; 40 had MS with ON (ON+), 40 had MS without ON (ON–) and 40 were healthy eyes (control group) (table 1).

OCT and OCT-angio parameters were summarised in table 2 and figures 1 and 2.

CMT, TMV, peripapillary RNFL, average GCC, superior GCC, inferior GCC, superficial layers of the foveal vessel density, superior peri and parafoveal sectors of the macular superficial vessel density, full foveal, perifoveal and parafoveal sectors vessel density all were affected with significant thinning affecting MS (ON+) and (ON–) patients compared with the control group. Best-corrected visual acuity (BCVA) was compared between the three studied groups and showed significant reduction among MS (ON+) and MS (ON–) compared with the control group (p<0.007). Post hoc test (TUKEY) was done for further comparison between the control group and each of the MS groups (online supplemental table 1).

PARP-1 gene expression level showed a significant difference comparing the three groups with the highest level being for the (ON+) group (p<0.0009), as shown in online supplemental table 3.

When the AUC values of the OCT variables were analysed for correct diagnostic power to distinguish between the eyes of healthy control and MS (ON+ and ON–), none of the parameters had the desired strong diagnostic power (all of AUC <0.5) in ROC curve analysis. The highest AUC value was observed in the CMT (AUC=0.329 (95% CI 0.71 to 0.80), and the second one was the average GCC (AUC=0.183, 95% CI 0.739 to 0.85), while the AUC value of the TMV was determined as 0.166 (95% CI 0.71 to 0.814). In contrast, when (AUC) values of PARP-1 gene expression were analysed for correct diagnostic power to distinguish between healthy control and MS (ON+ and ON–), the highest AUC value was 0.9 (95% CI 0.78 to 0.82). ROC curve analysis and AUC values are shown in figure 3.

Negative correlations were found between PARP-1 gene expression level and CMT (r=–0.3, p=0.001, TMV (r=–0.1, p=0.05) and full foveal vessel density thickness (r=–0.2, p=0.004).

PARP-1 gene expression level was further correlated to both groups of MS (ON+ and ON–) separately, and a significant negative corrleations were found between PARP-1 gene expression level and the following parameters in MS (ON+): CMT (r=–0.465, p=0.002), TMV (r=–0.420, p=0.007), temporal disc vessel density (r=–0.428, p=0.006) and BCVA (r=–0.414, p=0.008) (online supplemental table 2).

DISCUSSION

MS is a well-known, potentially blinding disease that causes multifocal zones of infiltrations by macrophages and T-lymphocytes, which lead to oligodendrocyte death and myelin sheath destruction with the formation of CNS plaques composed of inflammatory cells and their products, demyelinated and transected axons, and astrogliosis affecting both white and grey matter.[13]

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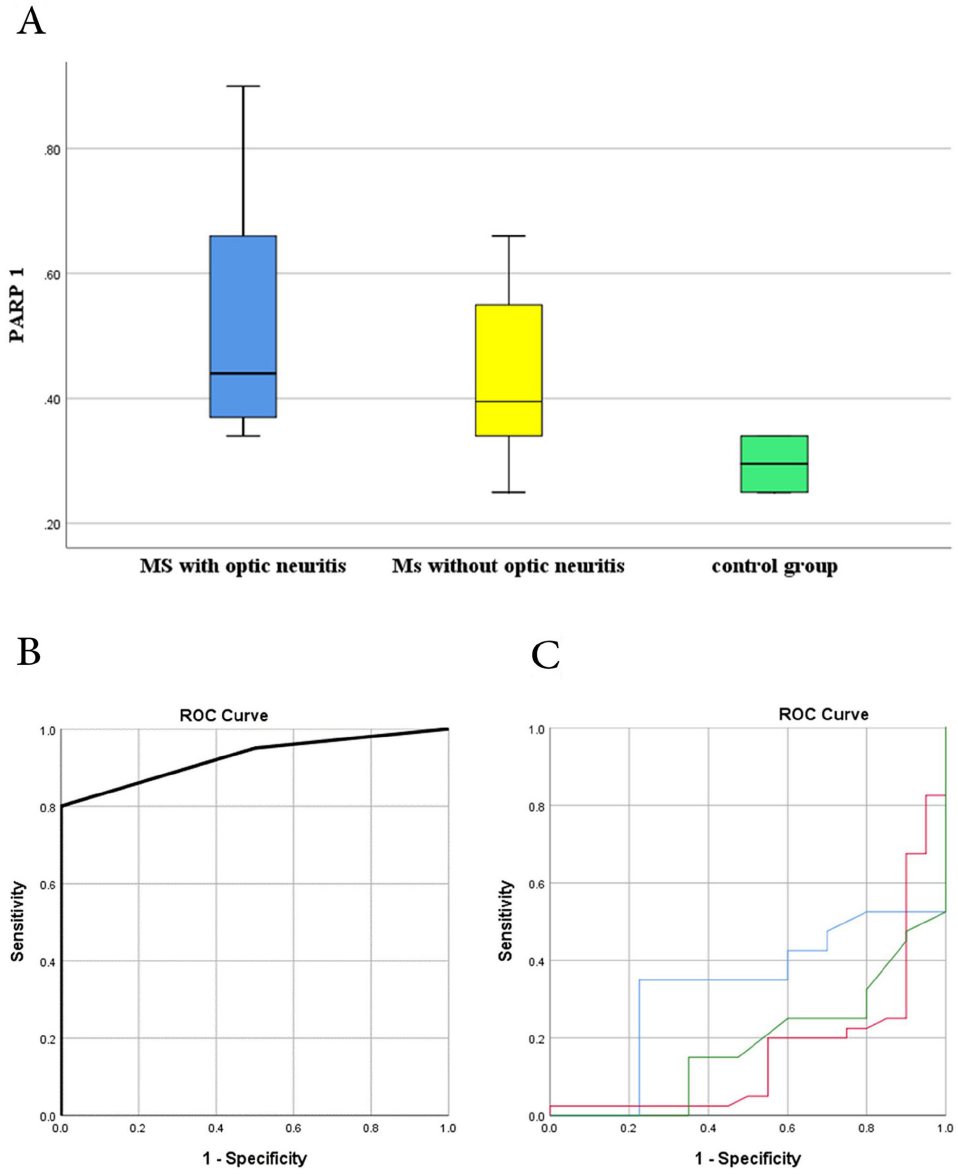

Figure 3 (A) Comparison between the three studied groups (ON+), (ON–) and control group regarding PARP 1 gene expression level. (B) ROC curve constructed by plotting the area under the receiver operating characteristic curve value is (0.9). (C) Rock curve constructed by plotting the area under the receiver operating characteristic curve value is: Blue line: Central macular thickness (0.329). Red line: total macular volume (0.166). Green line: average GCC (0.183). GCC, ganglion cell complex; ON, optic neuritis; ROC, receiver operating characteristic.

There was a significant difference in mean age among the three studied groups (table 1). This is consistent with previous reports that there is a wide range of age at onset (ranges from 15 to 60 years), with typically the most common age of onset (20–40 years).[14]

BCVA was compared between the three studied groups and showed significant reduction among MS (ON+) and MS (ON–) compared with the control group (p-value<0.007). It was proved that MS could be associated with poor recovery of BCVA due to neurodegenerative process that may cause dysfunctional mechanisms involving the foveal photoreceptors and bipolar cells.[15]

There was significant decrease in (CMT) in groups (A and B) compared with group C, which can be due to the preferential thinning of the central macula relative to the peripheral macular region in the eyes of patients with MS compared with normal eyes. The histological distribution of the nerve fibre layer and retinal ganglion cell in the macula, could be the cause of this macular thickness pattern. It seems to be particularly informative of neurodegeneration in the eyes of patients with MS whether they have a history of ON or not.[16]

We found a significant decrease in the TMV in groups (A and B) when compared with group C, which can be attributed to the analogies between the macular volume and CNS grey matter, as the macula consists of ~34% neuronal cells by average thickness in healthy eyes. Retrograde degeneration from lesions in the optic nerves, chiasm or tracts, could be the origin of the neuronal loss apperared as OCT- macular volume thinning in the eyes

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of MS measured by OCT. Those subclinical neurodegeneration findings may be started in eyes with MS even before ON attack, and may be used in the future as a predictive biomarkers of ON attack.[17]

There is thinning of the peripapillary (RNFL) thickness in both groups (ON+ and ON−) when compared with the healthy control group, which was consistent with Birkeldh *et al*.[18]

Average (GCC), superior (GCC) and inferior (GCC) were found to be thin when compared with the control group, which was also stated by Özbilen *et al*.[19]

OCT-A is considered a reliable marker of disease and disability in definite patients with MS.[20]

We found a marked thinning of the superficial and full macular capillary plexus thickness in both groups (ON+and ON−) compared with the control group. This finding is consistent with Rogaczewska *et al*, who stated that the superficial capillary plexus thickness was significantly lower in the eyes of patients with MS than in the controls.[21]

Peripapillary region vessel density reduction in the whole sectors, revealed through OCT-angio, is consistent with Feucht *et al*, who considered such reduction of blood supply correlated to tissue atrophy with subsequent reduction of their metabolic needs.[10]

However, our finding of vessel density reduction in both macula and peripapillary capillary plexus is consistent with Cennamo *et al* that the RNFL might suffer indirectly from vascular damage to the optic nerve, and the story might start as a vasculopathy ended by nerve fibre layer reduction.[22]

When the AUC values of the OCT variables was analysed for correct diagnostic power to distinguish the eyes of healthy control and definite MS (ON+ and ON−), the highest AUC value was observed in the CMT (AUC=0.329 (95% CI 0.71 to 0.80), and the second one was the average GCC (AUC=0.183, 95% CI 0.739 to 0.85), while the AUC value of the TMV was determined as 0.166 (95% CI 0.71 to 0.814). This finding was consistent with Lamirel *et al* and M. and Poplyak *et al*.[23 24]

However, none of the above-mentioned parameters had the desired strong diagnostic power (all of AUC<0.5) in ROC curve analysis. This finding was consistent with Özbilen *et al*,[19] however, this could also be attributed to the small sample size of the study.

PARP1 gene expression level was found higher in (MS ON+) compared with (MS ON−) and control groups. To further evaluate the diagnostic significance of PARP-1, the ROC curve was constructed by plotting the AUC value (AUC 0.9, 95% CI 0.78 to 0.82). In a study performed on peripheral blood monocytes of relapsing-remission patients with MS and secondary progressive MS (SPMS), Farez *et al* showed that PARP-1 enzymatic activity was significantly higher in patients with SPMS.[25]

The above-mentioned variables highlight the correct diagnostic power distinguishing patients with MS from a healthy control. We found that the OCT parameters are biomarkers of definite patients with MS who had previous attacks. They may be replaced by a more powerful and conclusive biomarker that may be even detected prior to opthalmic damage, the PARP-1 gene expression level.

We found a higher expression of PARP-1 patients with MS with ON compared with patients without ON and control groups. Luo *et al*[26] suggested that PARP-1 negatively regulates the immunosuppressive function of Treg cells at the post-translational level by way of FOXP3 poly (ADP-ribosyl)ation. Collectively, these data suggest a potentially important impact of the deregulation of the PARP-1/TGFBR axis on T-cell function in MS.[27]

We found a significant negative correlation between PARP1 gene expression level and CMT, TMV and full-thickness foveal vessel density in both groups of MS (ON+and ON−). PARP-1 gene expression level was further correlated to both groups of (MS ON+) and (MS ON−) and a significant negative corrleations were found between PARP-1 gene expression level and the following parameters in MS (ON+): CMT (r=−0.465, p=0.002),TMV (r=−0.420, p=0.007), Temporal disc vessel density(r=−0.428, p=0.006) and BCVA(r=−0.414, p=0.008). To the best of our knowledge, this is the first time to correlate PARP1 gene expression level to macular OCT and OCT-angio parameters.

It was proved that (PARP1) has a role in neurodegenerative diseases. PARP1 links to a cluster of stress signals arrised by inflammation, and excessive inflammation predisposes the vasculature to stiffening, dysfunction and alteration of endothelial function which will lead to chronic inflammation and tissue damage, which in case of MS disease neurosis may be the sign of such tissue damage.[28 29]

Limitations of the study: the small sample size. The evaluated macular area was different among GCC, vessel density at the four different levels, macular volume, full macular thickness and parafoveal and perifoveal sectors thickness.

In conclusion: PARP-1 may play an important role in the development of the ON cascade (excessive inflammation which lead to angiopathy and subsequent tissue damage and neurosis) in patients with MS and may be a biomarker for diagnosing and a potential molecular target of ON in MS patients' therapy. In addition to the OCT and OCT-angio changes that could be detected retrospectively, PARP-1 gene expression level could be considered a prospective detector to complete the full-blown picture of MS (ON+) early and prevent blindness. The diagnostic and prognostic values of PARP-1 and therapeutic applications are worth further investigation.

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Supplementary table (1): Post hoc analysis (TUKEY) test:

Dependent Variable			Mean Difference	Std. Error	Sig.
BCVA	control group	MS ON+	.59125*	.03003	.000
		MS ON-	.29500*	.03003	.000
CMT	control group	MS ON+	14.875*	4.288	.002
		MS ON-	5.975	4.288	.348
TMV	control group	MS ON+	.44425*	.06232	.000
		MS ON-	.28425*	.06232	.000
SS Peri -FVD	control group	MS ON+	2.250*	.721	.006
		MS ON-	2.300*	.721	.005
SN Para-FVD	control group	MS ON+	-1.500-	.926	.241
		MS ON-	-2.800-*	.926	.009
FFVD	control group	MS ON+	17.450*	3.857	.000
		MS ON-	5.800	3.857	.293
FS Para-FVD	control group	MS ON+	18.000*	4.747	.001
		MS ON-	12.100*	4.747	.032
FS Peri-FVD	MS ON-	MS ON+	17.350*	3.033	.000
		MS ON-	6.700	3.033	.074
FI Para-FVD	control group	MS ON+	23.575*	3.167	.000
		MS ON-	21.775*	3.167	.000
FI Peri- FVD	control group	MS ON+	19.084*	3.223	.000
		MS ON-	9.934*	3.223	.007
FN Para-FVD	control group	MS ON+	10.125	5.082	.119
		MS ON-	14.675*	5.082	.013
FN Peri-FVD	control group	MS ON+	16.171*	4.297	.001
		MS ON-	3.171	4.297	.741
FT Para–FVD	control group	MS ON+	19.375*	3.388	.000
		MS ON-	20.075*	3.388	.000
FT Peri-FVD	control group	MS ON+	21.650*	4.033	.000
		MS ON-	14.183*	4.356	.004
SRNFL	control group	MS ON+	7.650*	3.189	.047
		MS ON-	8.050*	3.189	.034
IRNFL	control group	MS ON+	15.925*	3.496	.000
		MS ON-	15.225*	3.496	.000
NRNFL	control group	MS ON+	9.100*	2.417	.001

		MS ON-	7.000*	2.417	.012
TRNFL	control group	MS ON+	11.300*	2.150	.000
		MS ON-	17.650*	2.150	.000
Average GCC	control group	MS ON+	11.125*	1.939	.000
		MS ON-	11.575*	1.939	.000
SGCC	control group	MS ON+	25.925*	8.833	.011
		MS ON-	24.675*	8.833	.017
IGCC	control group	MS ON+	10.975*	1.995	.000
		MS ON-	13.375*	1.995	.000
SDVD	control group	MS ON+	.575	.941	.814
		MS ON-	3.425*	.941	.001
IDVD	control group	MS ON+	1.075	1.305	.689
		MS ON-	3.175*	1.305	.043
NDVD	control group	MS ON+	.325	1.213	.961
		MS ON-	3.925*	1.213	.004
TDVD	control group	MS ON+	2.725*	1.120	.043
		MS ON-	1.125	1.120	.575
PARP 1	control group	MS ON+	-.23100-*	.02980	.000
		MS ON-	-.14300-*	.02980	.000

CMT (central macular thickness), TMV (total macular volume), SS Peri-FVD (superficial superior peri-foveal vessel density, SN Para-FVD (superficial nasal para-foveal vessel density), FFVD (full foveal vessel density), FS Para-FVD (full superior para-foveal vessel density), FS Peri-FVD (full superior peri-foveal vessel density), FI Para-FVD (full inferior para-foveal vessel density), FI-Peri FVD (full inferior peri-foveal vessel density), FN Para-FVD (full nasal para foveal vessel density), FN Peri-FVD (full nasal peri-foveal vessel density), FT Para-FVD (full temporal para-foveal vessel density), FT Peri-FVD (full temporal peri-foveal vessel density), SRNFL (superior peri-papillary retinal nerve fiber layer thickness), IRNFL (inferior peri-papillary retinal nerve fiber layer thickness), NRNFL (nasal peri-papillary retinal nerve fiber layer thickness), TRNFL (temporal peri-papillary retinal nerve fiber layer thickness), Average GCC (average ganglion cell complex thickness), SGCC (superior ganglion cell complex thickness), IGCC (inferior ganglion cell complex thickness), SDVD (superior disc vessel density), IDVD (inferior disc vessel density), NDVD (nasal disc vessel density), TDVD (temporal disc vessel density), PARP1 (Poly(ADP-ribose) Polymerase-1).

Supplementary 2: Bivariate correlation between PARP 1 levels and other parameters in both groups

	Ms with optic neuritis		Ms without optic neuritis	
PARP-1 with the following parameter	(r)	P	(r)	P
CM thickness	-.465	.002	-.065	.690
TMV	-.420	.007	.143	.380
Average GCC	.443	.004	.269	.094
TDVD	-.428	.006	-.081-	0.6
BCVA	-0.414	0.008	-0.17	0.27

CMT (central macular thickness), TMV (total macular volume), Average GCC (ganglion cell complex), TDVD (temporal disc vessel density), BCVA (best corrected visual acuity)

Supplementary 3: Comparison between the studied group regarding PARP 1 levels

	MS (ON+) N=40		MS (ON-) N=40		Control N=40		P value
	Mean	SD	Mean	SD	Mean	SD	
PARP1	**.53**	**.18**	**.44**	**.14**	**.30**	**.05**	**<0.0009**

*p-value was calculated by one-way ANOVA test