Original Article

Clinicopathological correlation of parapapillary atrophy in monkeys with experimental glaucoma and temporary central retinal artery occlusion

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Objective: To investigate the clinicopathological correlation of parapapillary atrophy. **Materials and Methods:** The study included 16 eyes of rhesus monkeys (*Macaca mulatta*) – 4 eyes with experimental glaucoma, 11 eyes after experimental temporary occlusion of the central retinal artery, and 1 normal eye. On histological sections, we measured zones with different histological characteristics.On fundus photographs, alpha zone and beta zone of parapapillary atrophy were measured and correlated with the histological data. **Results:** The size of the clinical alpha zone of parapapillary atrophy was significantly correlated with the size of the histological region with irregularities of the retinal pigment epithelium ($P = 0.05$; correlation coefficient $r = 0.49$) and with the size of the histological region with a decreased density of retinal photoreceptors ($P = 0.01$; $r = 0.60$). The size of clinical beta zone of parapapillary atrophy significantly correlated with the size of the histological region with complete loss of the retinal pigment epithelium ($P < 0.001$; $r = 0.91$), with the size of the histological zone with a complete loss of photoreceptors ($P < 0.001$; $r = 0.81$), and with the size of the histological zone with a closed choriocapillaris ($P < 0.001$; $r = 0.89$). **Conclusions:** The clinically seen alpha zone of parapapillary atrophy correlates with histological parapapillary irregularities of the retinal pigment epithelium and decreased density of retinal photoreceptors. The clinically seen beta zone of parapapillary atrophy correlates with histological complete loss of the retinal pigment epithelium and of the photoreceptors, and a closure of the choriocapillaris.

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Glaucomatous optic neuropathy is associated with a number of morphologic changes in the optic disc, retina, and choroid. These include loss of neuroretinal rim, deepening of the optic cup, splinter-shaped disc hemorrhages, localized and diffuse diminution of retinal nerve fiber layer, diffuse and focal narrowing of retinal arterioles, and parapapillary chorioretinal atrophy.[1] Parapapillary atrophy has been divided into a peripheral alpha zone characterized by irregular pigmentation and a beta zone bordering the optic disc border and showing visible sclera and large choroidal vessels upon ophthalmoscopy.[2-7]Although several hospital-based studies, population-based investigations, and experimental studies have shown the association of beta zone of parapapillary atrophy with glaucoma, there is only scarce information about the histology of parapapillary atrophy.[8-10] Better knowledge about clinicopathological correlation of the alpha zone and beta zone of parapapillary atrophy may help to elucidate the pathogenesis of parapapillary atrophy – the etiology of which has remained mostly unclear so far. We therefore conducted this study to examine the histology of parapapillary atrophy in a clinicopathological correlation in experimental glaucoma and to compare that seen in experimental temporary occlusion of the central retinal artery (CRAO) and a normal monkey eye. The study included monkey eyes with CRAO since it was not the purpose of the investigation to assess potential differences in parapapillary atrophy between glaucomatous eyes and eyes with CRAO but to get more information on the direct clinical-pathological correlation of parapapillary atrophy in eyes with different types of optic nerve damage.

Materials and Methods

The study comprised 16 eyes of rhesus monkeys (*Macaca mulatta*). At Iowa City, USA, experimental glaucoma had been unilaterally produced by multiple applications of argon laser to the trabecular meshwork and temporary CRAO by transient clamping of the CRAO at its site of entry into the dural sheath of the optic nerve.[4,11] The methods of producing experimental glaucoma or temporary CRAO have been described in detail previously.[4,11] The contralateral eyes were not available for this study because they had already been used for other earlier studies.[12,13] The study design was complied with the National Institute of Health's as well as University of Iowa's Institutional Guidelines for the Care and Use of Laboratory Animals.

All animals were serially examined under ketamine anesthesia (8-10 mg/kg body weight) before and during follow-up of the induction of CRAO or glaucoma. These examinations included intraocular pressure measurement by Goldmann applanation tonometry, ophthalmoscopic examination, and color fundus photography. The intraocular pressure measurements by Goldmann applanation tonometry were performed serially at the beginning of the study and once the eyes developed elevated intraocular pressure (sustained intraocular pressure of >21 mmHg). Before the

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application of laser photocoagulation, intraocular pressure was measured three times on 3 different days to establish a baseline for each eye. During the follow-up period, the frequency of intraocular pressure measurements depended upon the level of intraocular pressure in each eye; the higher the intraocular pressure, the more frequently it was measured, i.e. 2-3 times a week when the intraocular pressure was >60 mmHg, weekly for intraocular pressure in the forties or fifties, and monthly for intraocular pressures of <40 mmHg. The objective was to maintain an intraocular pressure between 30 and 40 mmHg to mimic the clinical situation of moderate ocular hypertension. Since with the argon laser trabecular application it is impossible to achieve a desired level of intraocular pressure on a long-term basis, the intraocular pressure often tended to go higher than the desired levels if left alone. Therefore, to maintain our desired level of intraocular pressure (i.e. 30-40 mmHg), ocular hypotensive drops were applied. Before sacrificing the animals, fundus photographs were taken. The enucleated globes were immediately placed in 4% buffered formalin. They were fixed for no less than 72 h, following which they were opened in a standard horizontal pupil–optic nerve plane, thereby incorporating the macular region, along with the optic nerve. The preparation of the globes was identical for all three study groups. The globes were prepared in routine manner for light microscopy.The cut anterior–posterior segments of the globe, going through the pupil and the optic nerve, were dehydrated in alcohol, imbedded in paraffin, sectioned for light microscopy, and stained by the Periodic acid-Shiff (PAS) method. For all eyes, one section running through the central part of the optic disc was selected for further evaluation. The histological slides were digitized and morphometrically analyzed using a microscope with built-in software for planimetric measurements (LEICA IM50 1.20, Leica Microsystems AG, Heerbruck, Switzerland). We measured the following at the optic disc border:

- the length of Bruch's membrane with an irregular shape and pigmentation of the retinal pigment epithelium [Fig. 1].
- the length of Bruch's membrane devoid of retinal pigment epithelium cells;
- the length of Bruch's membrane with an apparently reduced density of retinal photoreceptors;
- the length of Bruch's membrane without adjacent retinal photoreceptors; and
- the length of Bruch's membrane with the underlying choriocapillaris closed.

The ophthalmoscopic evaluation of the optic disc was performed by morphometric assessment of fundus photographs taken at the end of follow-up.The disc slides were projected in a scale of 1-15. The outlines of the optic cup, optic disc, peripapillary scleral ring, and alpha zone and beta zone of the parapapillary atrophy were plotted on paper and morphometrically analyzed.The border of the optic disc was identical with the inner side of the peripapillary scleral ring. The parapapillary atrophy was differentiated into a peripheral alpha zone with irregular pigmentation and a central beta zone with visible Bruch's membrane and visible large choroidal vessels. The method has already been described in detail.[2] Since the magnification of the optic disc photographs varied according to the period of the study and the fundus camera used, all disc measurements of the same monkey were adapted to the photographic magnification of the first photograph. Because keratometric readings and refractometry had not been performed for all monkeys included in the study, the optic disc measurements were expressed in relative size units. The photographs were evaluated in a masked fashion by a single examiner (JBJ) without knowledge of the diagnosis or treatment. Technical reasons prevented the intrapapillary region from being covered during the assessment of the parapapillary atrophy. The retinal nerve fiber layer was semiquantitatively assessed in a scale ranging between "0" for "no visibility" and "64" for "extreme nerve fiber layer visibility" in all quadrants.

Statistical analysis was performed by using a commercially available statistical software package (SPSS for Windows, version 17.0, SPSS, Chicago, IL, USA). The data were presented as means and standard deviations as well as medians and ranges. For the comparison of the study groups, statistical tests (Mann–Whitney test or Student's *t*-test) for unpaired samples were applied. For the comparison of parameters within the same optic nerve head, statistical tests (Wilcoxon test or Student's *t*-test) for paired samples were applied. If there were two or more variables associated with the dependent parameter, a multivariate analysis was performed. 95% confidence intervals (CI) were presented. The level of significance was 0.05 in all statistical tests.

Results

The study included 16 eyes of 16 animals with a mean age of 17.6 ± 6.5 years (mean ± standard deviation; range: 13-24 years). The total study group comprised 4 eyes with experimental glaucoma [age: 14 ± 1 years (13, 13, 15, and 15 years)], 11 eyes after experimental temporary CRAO (age: 17 ± 5 years), and 1 normal eye (age: 24 years).

The temporary CRAO had been produced by clamping of the CRAO for 97-240 min (mean: 150 ± 38 min). The interval between temporary induction of CRAO and sacrificing the animals was 112 ± 111 days (median: 67 days; range: 36-351 days). The experimental glaucoma had been produced 30 ± 15 months (range: 7-41 months) prior to sacrificing the animals.

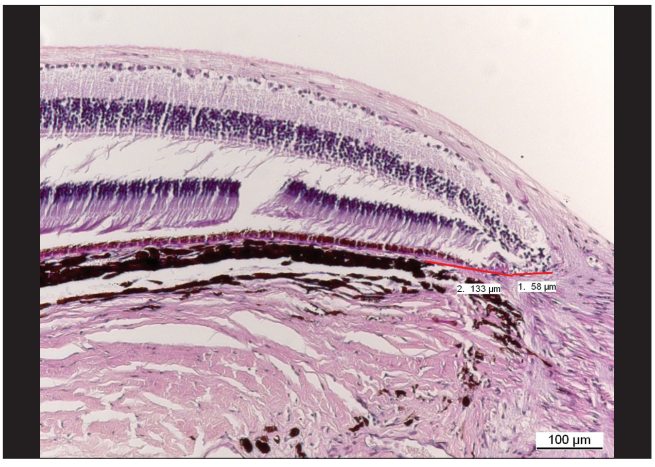

Figure 1: Microphotograph of the optic nerve head showing the histological zone of irregular pigmentation of the retinal pigment epithelium. The region between the two red vertical bars extended to the level of the retinal pigment epithelium is the region of irregular pigmentation of the retinal pigment epithelium

In the glaucoma group, the intraocular pressure measured under ketamine anesthesia at follow-up examinations was 24 ± 12 mmHg. The intraocular pressure was in the normal range in the normal eyes and the eyes with CRAO. In the eyes of the glaucoma group, the objective was to maintain an intraocular pressure between 30 and 40 mmHg. Since with the argon laser trabecular application, it was impossible to achieve the desired level of intraocular pressure on a long-term basis, the intraocular pressure often tended to go higher than the desired levels if left alone. Therefore, to maintain our desired level of intraocular pressure (i.e. 30-40 mmHg), we had to use ocular hypotensive drops, such as topical betabockers and miotics, in 90% of the glaucoma eyes. At the end of the study period, the visibility of the retinal nerve fiber layer was completely lost in three animals of the glaucoma group, and in one animal of the glaucoma group, the nerve fiber layer visibility was reduced to a score of about 25% of the maximal score.

The size of the alpha zone of parapapillary atrophy, as measured on the fundus photographs, was significantly correlated with:

- the size of the histological region with irregularities of the retinal pigment epithelium ($P = 0.05$; standardized correlation coefficient $\beta = 0.49$; non-standardized regression coefficient B = 0.002; 95% CI: 0.000, 0.004) [Fig. 2] and
- the histological zone with a decreased density of retinal photoreceptors ($P = 0.01$; $\beta = 0.60$; B = 0.004; 95% CI: 0.001, 0.006) [Fig. 3].

The size of the alpha zone of parapapillary atrophy as measured on the fundus photographs was statistically significantly associated neither with the size of the histological zone with a closed choriocapillaris ($P = 0.94$) nor with the histological zone with a complete loss of photoreceptors ($P = 0.87$).

The size of the beta zone of parapapillary atrophy, as measured on the fundus photographs, was significantly correlated with:

- the size of the histological region with a complete loss of the retinal pigment epithelium ($P < 0.001$; $\beta = 0.91$; B = 0.002; 95% CI: 0.002, 0.003);
- the size of the histological zone with a complete loss of photoreceptors ($P < 0.001$; $\beta = 0.81$; B = 0.003; 95% CI: 0.002, 0.005) [Fig. 4]; and
- the size of the histological zone with a closed choriocapillaris ($P < 0.001$; $\beta = 0.89$; B = 0.003; 95% CI: 0.002, 0.003) [Fig. 5].

The size of the clinical beta zone was not statistically significantly associated with the size of the histological zone with a reduced density of photoreceptors ($P = 0.24$). The association between the size of the histological zone with a complete loss of photoreceptors and the size of the beta zone of parapapillary atrophy [Fig. 4] remained statistically significant if the outlier in upper right end of the scattergram was excluded ($P = 0.04$; $\beta = 0.52$; B = 0.001; 95% CI: 0.001, 0.002).

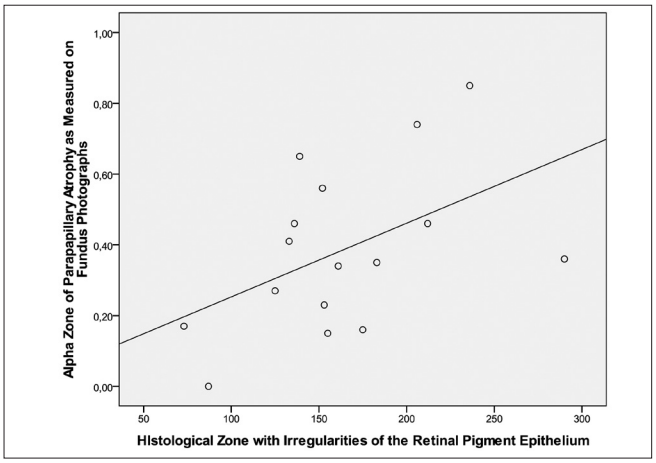

Figure 2: Scattergram showing the relationship between the size of the histological zone of parapapillary Bruch's membrane with irregularities in the retinal pigment epithelium and the size of the clinical alpha zone of parapapillary atrophy. The correlation was statistically significant ($P = 0.05$; correlation coefficient = 0.49)

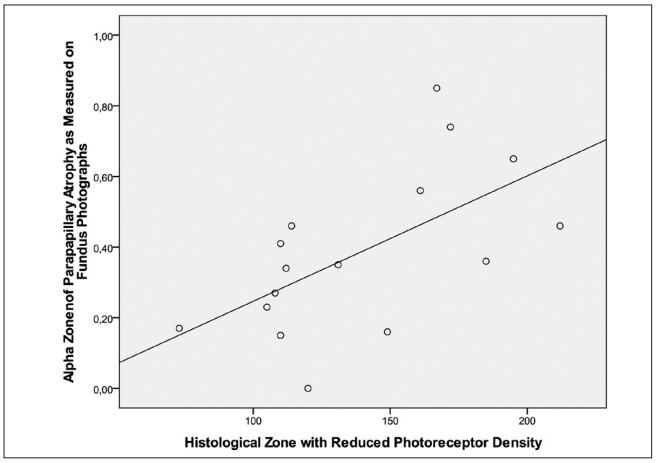

Figure 3: Scattergram showing the relationship between the size of the histological zone of parapapillary Bruch's membrane with a reduced density of retinal photoreceptors and the size of the clinical alpha zone of parapapillary atrophy. The correlation was statistically significant ($P = 0.01$; correlation coefficient = 0.60)

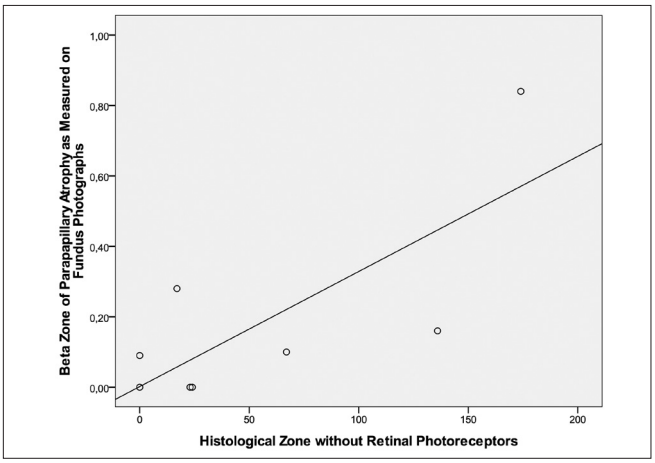

Figure 4: Scattergram showing the relationship between the size of the histological zone of parapapillary Bruch's membrane without retinal photoreceptors and the size of the clinical beta zone of parapapillary atrophy. The correlation was statistically significant ($P < 0.001$; correlation coefficient = 0.81)

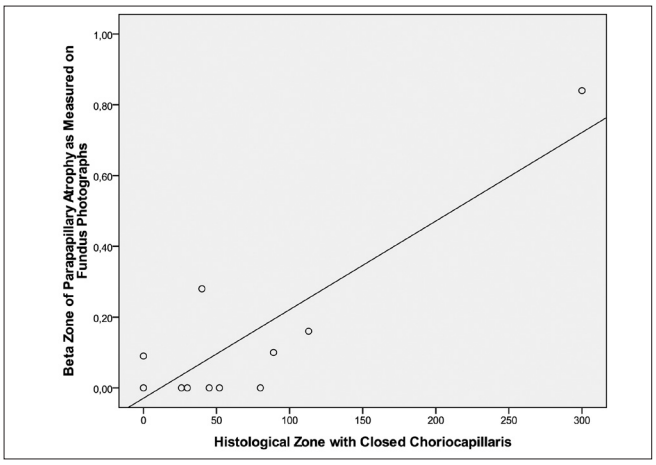

Figure 5: Scattergram showing the relationship between the size of the histological zone of parapapillary Bruch's membrane with the choriocapillaris closed and the size of the clinical beta zone of parapapillary atrophy. The correlation was statistically significant ($P < 0.001$; correlation coefficient = 0.89)

Statistical comparison between the glaucoma group and the occlusion of the central retinal artery group

Although the three study subgroups (4 eyes with experimental glaucoma, 11 eyes after experimental temporary CRAO, and 1 normal eye) were relatively small for a statistical comparison between them, the following were significantly or marginally significantly larger in the glaucoma group than in the CRAO group: Beta zone of parapapillary atrophy (0.28 ± 0.38 units vs. 0.01 ± 0.3 units; $P = 0.04$), the histological zone without retinal pigment epithelium cells (128 ± 154 units vs. 20 ± 33 units; $P = 0.04$), the histological zone without retinal photoreceptors (94 ± 77 units vs. 4 ± 10 units; $P = 0.04$), and the region with a closed choriocapillaris (126 ± 126 units vs. 21 ± 28 units; $P = 0.07$). The glaucoma group and the group with experimental temporary CRAO did not vary significantly in the size of alpha zone of parapapillary atrophy (0.40 ± 0.05 units vs. 0.34 ± 0.23 units; $P = 0.41$), the histological zone with irregular retinal pigment epithelium (186 ± 73 units vs. 149 ± 43 units; $P = 0.66$), and the histological zone with a reduced density of retinal photoreceptors (135 ± 35 units vs. 138 ± 43 units; $P = 0.85$).

Discussion

The results indicate that there is a correlation between histological and clinical parameters of:(a) the alpha zone of parapapillary atrophy and parapapillary irregularities of the retinal pigment epithelium and a decreased density of retinal photoreceptors and (b) the beta zone of parapapillary atrophy and a complete loss of the retinal pigment epithelium, a complete loss of photoreceptors, and a closure of the choriocapillaris.

Our study agrees with previous investigations.Fantes and Anderson described that a choriosclera crescent occurred when the retinal pigment epithelium was retracted from the disc margin, which was most prominent when associated with a tilted exit canal for the axon bundles through the sclera. In such a crescent, the choroid was thinned or absent next to the disc, exposing to view some of the underlying sclera.[8] In another study, the alpha zone correlated with irregularities of the retinal pigment epithelium and beta zone with a complete loss of retinal pigment epithelium cells and a loss of adjacent retinal photoreceptors.[9] In still another study, the clinical beta zone showed a complete loss of retinal pigment epithelium cells and an incomplete loss of adjacent photoreceptors, and the alpha zone showed irregularities in the retinal pigment epithelium.[10] However, compared with the above studies, our study additionally showed that the clinical alpha zone is associated with a decreased density of photoreceptors and that the clinical beta zone additionally showed a closure of the choriocapillaris. Our study is in agreement with a recent clinical psychophysical examination in which the size of the blind spot of the visual field was measured by direct fundus microperimetry.[14] In that investigation, alpha zone was associated with a relative scotoma, corresponding to a partial loss of retinal pigment epithelium cells and photoreceptors as found in the present study, and the clinical beta zone was associated with an absolute scotoma, corresponding with the complete loss of photoreceptors and retinal pigment epithelium cells and the closure of the choriocapillaris as detected in the present study.

The finding of our study on a closure of the choriocapillaris in the parapapillary region agrees with other experimental, clinical, and histopathologic studies on the parapapillary atrophy in glaucoma. Fluorescein angiographic studies in experimentally raised intraocular pressure in eyes of monkeys showed that the parapapillary choroid was more susceptible to obliteration by the raised pressure than the rest of the choroid.[15-17] Similarly, Kalvin and colleagues, on latex injection with raised intraocular pressure in monkeys, found poor filling primarily in the peripapillary choroid.[18] De Freitas and Morin studied 25 glaucomatous albino rabbits with raised intraocular pressure, after injection of India ink or latex, and found parapapillary choroidal filling defects of variable size in all glaucomatous eyes.[19] In clinical studies, Laatikainen described that 60% of glaucomatous eyes with moderately controlled intraocular pressure showed delayed or deficient filling of the peripapillary choriocapillaris.[20] Boyd and Rosen, in half of their glaucomatous eyes, found on fluorescein angiography a filling defect in the parapapillary choroid.[21] They found a measurable "circumpapillary choroidal perfusion delay" in all eyes with glaucoma, which could be improved on lowering the intraocular pressure. Similar peripapillary choroidal delay or filling defects have been reported by other workers in patients with glaucoma and low-tension glaucoma, as well as seen in a study of these eyes by one of us (personal communication).[22] In histopathologic studies, a particularly severe reduction in the number and size of vessels in the parapapillary choroid and parapapillary choriocapillaris atrophy have been reported in glaucomatous eyes.[23,24]

Potential limitations of our study should be mentioned. First, in an investigation with a study design as in ours, a potentially very helpful strategy would have been to perform a bilateral comparison. Since, however, the contralateral eyes were not available in our study, this potentially very useful strategy could not be followed.Second, the number of monkeys included in the study was relatively small. It could be argued that, for example, only four eyes with experimental glaucoma would be insufficient to provide a broad range of information to cover early and late disease. Three of these four eyes showed advanced glaucomatous optic nerve damage, and the third

eye had a medium advanced damage, as demonstrated by the reduced visibility of the retinal nerve fiber layer.Despite the low number of monkey eyes with glaucoma, however, the correlations were statistically significant, strengthening the validity of the results and conclusions. The single normal eye did not serve to be compared with the eyes of the glaucoma group and the eyes of the CRAO group, but with all eyes together served to elucidate the histopathologic correlate of parapapillary atrophy. Third, the non-glaucomatous group of eyes was mainly composed of eyes after CRAO. Since, however, it was not the primary goal of our study to compare the prevalence of parapapillary atrophy between glaucomatous monkey eyes and normal monkey eyes but to get information about the histology of parapapillary atrophy, the overall composition of the whole study population may not have influenced the results and conclusions of our study.

In conclusion, our experimental studies in monkeys on clinicopathological correlation of parapapillary atrophy show that the clinically seen alpha zone of parapapillary atrophy correlates with histological parapapillary irregularities of the retinal pigment epithelium and decreased density of retinal photoreceptors. The clinically seen beta zone of parapapillary atrophy correlates with histological complete loss of the retinal pigment epithelium and of the photoreceptors, and a closure of the choriocapillaris.

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