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·Clinical Research·

Presence of Fleischer ring and prominent corneal nerves in keratoconus relatives and normal controls

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Abstract

• AIM: To examine the occurrence of commonly known clinical signs of keratoconus (KC), *i.e.* Fleischer ring, prominent corneal nerves and thinning, among unaffected family members of KC patients and healthy control individuals.

• METHODS: Data of both eyes of 117 relatives of KC patients having no manifest disease based on videokeratography indices (KC relatives), and 142 controls were used for Pearson correlation and *t*-test statistics. Correlation of Fleischer ring, prominent corneal nerves and central pachymetry data were tested with each other and with videokeratography indices (KSI, KISA, 3 and 6 mm Fourier asymmetry, and I-S).

• RESULTS: A moderate correlation was found between Fleischer ring and all examined topographical indices. Most important correlation was present with 6 mm Fourier asymmetry, and corneal pachymetry (r=0.272, P<0.001; r=-0.234, P=0.027, respectively). Similar correlations were found with prominent corneal nerves (r=0.234, P<0.001 for 6 mm Fourier asymmetry and r=-0.235, P=0.0265 for pachymetry). KC family members who exhibited Fleischer ring or prominent nerves had thinner and more asymmetric corneas than those without Fleischer ring or prominent corneal nerves (P<0.05 for pachymetry and topographic indices with *t*-test and Mann-Whitney rank sum test). Though rarely, Fleischer ring and prominent corneal nerves occurred among normal controls, indicating the existence of forme fruste cases in the normal population. Control subjects, who had corneal Fleischer ring or prominent nerves had corneas more similar to KC than other controls (*t*-test: increased KSI and KISA, P=0.048 and 0.012, respectively).

• CONCLUSION: In KC family members and healthy individuals, Fleischer ring and prominent corneal nerves are associated with features of KC and may suggest a possibility of forme fruste KC. Searching for the possible presence of Fleischer ring or prominent nerves on the cornea may help in the decision whether or not to diagnose subclinical KC in a borderline case.

• **KEYWORDS:** forme fruste/subclinical keratoconus; Fleischer ring; corneal nerves; corneal thinning; videokeratographic indices; iatrogenic keratectasia

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INTRODUCTION

Keratoconus (KC, OMIM #148300) is a progressive, bilateral, primary ectatic disease of the cornea, characterized by non-inflammatory thinning and conical protrusion [1]. In the general population, its estimated prevalence lies between 50 and 230 per 100 000 [2], but depending on diagnostic criteria and ethnicity, it can reach 2.34%[3]. KC is frequently an isolated condition, but it may associate with other systemic or ocular disorders [1,4]. There is a well-recognised genetic component to KC, as evidenced by twin [5] and family studies [1,6-8]; however, the etiology of the disease is complex, multifactorial with both genetic and environmental factors playing a role[9,10].

Amsler [11] demonstrated similar but less pronounced corneal topographical changes by Placido disc in relatives of KC patients, and called this quite frequent phenomenon forme fruste KC. He proved that classic and forme fruste KC constituted the same entity and that must be considered in genetic studies. Topographic examination of relatives of KC patients revealed aggregation of asymmetric corneal patterns and increased videokeratoscopy indices [7,12]. Topographic features similar to forme fruste KC were observed among some refractive surgery candidates, especially among the ones suffering from progressive keratectasia after the intervention [13]. Relatives of patients with KC have a high prevalence of undiagnosed KC and KC traits, therefore, keratorefractive surgery should be considered cautiously in these individuals[14,15].

KC severity was found to be associated with an increasing rate of slit-lamp biomicroscopic signs, *i.e.* Vogt striae,

Table 1 Characteristics of the examined 47 KC families and control individuals

KC families (n=47)	KC patients	KC family members	Healthy controls[c]
No. of subjects	49 (87 eyes) [a]	117 (233 eyes) [a]	142 (279 eyes) [b]
Age (a): $\bar{x} \pm s$ (range)	33.3 ± 11.7 (15-64)	41.3 ± 18.6 (9-81)	35.9 ± 13.7 (14-74)
No. of subjects having additional central corneal pachymetry measurements	15 (27 eyes)	45 (89 eyes)	26 (52 eyes)
No. of subjects having Fleischer ring	33 (71.7%)	36 (30.8%)	3 (2.1%)
No. of subjects having prominent corneal nerves	7 (15.2%)	17 (14.5%)	4 (2.8%)

All subjects underwent slit-lamp and topographic examination, and a subpopulation had additional central corneal pachymetry measurements. [a] In two families, two members were affected. Data of operated eyes were excluded (3 and 5 probands underwent bilateral and unilateral keratoplasty, respectively, and one relative had previous eye injury); [b]Five eyes having previous injuries were excluded; [c]Controls were not significantly different from the members of KC families, concerning age (P=0.11) and gender distribution (P=0.153).

F leischer ring, prominent nerves, haze and thinning of the cornea. Fleischer ring is present in most KC corneas whereas prominent nerves are less frequent and specific for KC[1,16].

Diagnosing forme fruste or subclinical KC is still challenging in many cases. However, identifying such cases is necessary both for genetic studies and screening potential high risk refractive surgery candidates. In our previous work, we observed the occurrence of Fleischer ring and prominent corneal nerves in unaffected KC family members [9]. Forme fruste KC is known to occur frequently among KC family members, therefore many studies examine KC family members to define subtle characteristics associated with forme fruste or subclinical KC [15,17]. In the present study, we investigated the correlation of corneal Fleischer ring and prominent nerves with videokeratographic signs of KC both among unaffected family members and healthy control individuals to find out if these signs can be used to better identify forme fruste KC.

SUBJECTS AND METHODS

Subjects Our study's design followed the tenets of the Helsinki Declaration with institutional research ethics board approval, informed consent of patients and controls was obtained before examination. KC families were recruited by mailing letters to patients treated in our department for KC, informing them about the aims of the study and asking for participation of the relatives, too. Healthy controls were recruited from students, personnel and patients of the department with healthy corneas. All of the enrolled subjects were Hungarian patients with Caucasian ancestry. Table 1 shows the groups of individuals enrolled in the study. Non-affected KC family members were defined as having ≤ 3 D astigmatism or ≤6 D spherical refractive error and not having KC based on keratoconus severty index (KSI) or KISA (values below 30% and 100%, respectively). None of the family members had any of the known genetic associations of KC (*e.g.* Down syndrome, Marfan syndrome *etc.*). Of 142 control individuals without family history of KC and without history of corneal disease or eye surgery were selected, with ≤3 D astigmatism or ≤6 D spherical refractive error. Only eyes that had no previous corneal injury and surgery, including perforating keratoplasty, were involved in the study.

Methods For each KC proband, diagnosis was based on clinical examination, including measurement of visual acuity, slit-lamp biomicroscopy, direct ophthalmoscopy, retinoscopy and videokeratography.

All KC family members and control individuals consented corneal topography, slit-lamp clinical examination, and 86 consented ultrasonic pachymetry (27 eyes of 15 KC patients, 89 eyes of 45 family members and 52 eyes of 26 controls; Table 1).

By slit-lamp biomicroscopy, the presence of Fleischer ring in the epithelium, prominent corneal nerves were searched for in family members of KC patients and controls. Fleischer ring was considered as present when at least 2 mm curved paracentral subepithelial iron deposit (incomplete) or a complete Fleischer ring was detected (Figure 1).

Videokeratography Topographic examination was performed on both eyes of each study participant, using a TMS-4 videokeratoscope (Tomey Corporation, Nagoya, Japan). At least four images per eye were taken, discarding those that were misaligned, out of focus or of poor quality. Care was taken to avoid artifacts during the examination which could be responsible for pseudokeratoconus images[18]. Data obtained from the best image of each eye, selected upon the quality of the keratoscopy mires by visual inspection, were used. Topographical images and parameters of operated eyes were ignored.

Videokeratography indices of keratoconus Videokeratography indices yielded by the TMS-4 software and those calculated from them were selected for further analysis.

KISA Values of KISA above 100 were taken to indicate manifest KC, below 60 healthy eyes, and the interval between the two being considered forme fruste cases[19].

Keratoconus severity index KSI expresses the severity of the disease in percentage, *i.e.* KC suspect >15%, manifest KC >30%[20].

Three and six milimeter Fourier asymmetry indices Decentration (first harmonic) component, on mire rings 1-9,

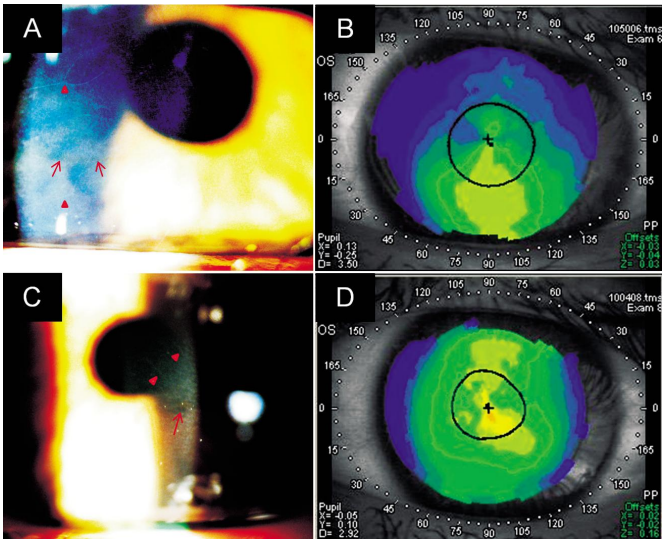

Figure 1 Slit-lamp and videokeratographic images of two healthy control individuals A: Slit-lamp photography of a 39 year-old man who has corneal Fleischer ring (arrows) and prominent nerves (arrowheads); B: Videokeratographic image of the same man shows asymmetric corneal pattern; C: Slit-lamp photography of a 32 year-old woman who has corneal iron deposition, which did not reach 2 mm length, therefore it was not considered as Fleischer ring (arrow), but she has prominent corneal nerves (arrowheads); D: Videokeratographic image of the same woman shows asymmetric corneal pattern with skewed radial axis.

and rings 1-20 which approximately represent the central 3 or 6 mm of the cornea[21,22].

I-S Absolute values of the I-S (inferior-superior dioptric asymmetry)[23]. [I-S]≥1.4 was considered abnormal.

Corneal pachymetry Corneal ultrasonic pachymetry was used to measure the central corneal thickness on both eyes of 86 individuals: 15 KC probands, 45 non-affected relatives and 26 controls (Table 1).

Statistical Analysis For calculating statistics, data of both the right and left eyes of each individual were included. In KC family members, 3 series of correlation statistics were tested: the presence of Fleischer ring, central corneal pachymetry data and the presence of prominent corneal nerves, with one another and with videokeratography indices. Correlation statistics were not performed in control individuals because the low number of cases with Fleischer ring or prominent corneal nerves. The presence of Fleischer ring and prominent nerves in the cornea were binary variables. Central corneal pachymetry data and all videokeratography indices were continuous variables. Correlation between variables was calculated using Pearson correlation statistic, P value and the correlation coefficient (r) were determined. Parameter values between groups were compared with t-test or Mann-Whitney rank sum test. The program SigmaStat 3.5 version was used for statistics and α=0.05 was the level of significance.

Table 2 Correlation statistics in KC relatives

Parameters	Pearson correlation	
	r	P
Fleischer ring		
Pachymetry	-0.234	0.027
KSI	0.169	0.00958
KISA	0.155	0.0178
3 mm Fourier asymmetry	0.214	0.001
6 mm Fourier asymmetry	0.272	0.0000261
I-S	0.229	0.000419
Pachymetry		
Prominent nerves	-0.235	0.0265
KSI	-0.377	0.000271
KISA	-0.342	0.00104
3 mm Fourier asymmetry	-0.425	0.0000328
6 mm Fourier asymmetry	-0.427	0.0000303
I-S	-0.338	0.00119
Prominent nerves		
Fleischer ring	0.00437	0.947
KSI	0.237	0.000261
KISA	0.246	0.000148
3 mm Fourier asymmetry	0.228	0.000463
6 mm Fourier asymmetry	0.234	0.000308
I-S	-0.0287	0.663

The presence of Fleischer ring in KC relatives showed significant correlation with central corneal pachymetry data and all topographic indices. Central corneal pachymetry data displayed significant correlation with the presence of prominent nerves and all topographic indices. The presence of prominent corneal nerves showed significant correlation with all topographic indices except for I-S. r: Correlation coefficient.

RESULTS

Table 1 shows the characteristics of the examined population. Controls were not significantly different from the members of KC families, concerning age (P=0.11) and gender distribution (P=0.153). In KC patients, Fleischer ring was detected in 71.7%, and prominent nerves of the cornea in 15.2%. In KC family members, corneal Fleischer ring was observed in 30.8% and prominent nerves were detected in 14.5%.

Among control individuals, Fleischer ring and prominent nerves were not completely absent but occurred in 2.1% and 2.8%, respectively (Table 1; Figure 1).

Correlation statistics in KC relatives among KC family members, there was a significant negative correlation between central corneal pachymetry and Fleischer ring (r= -0.234; Table 2). For each topographic index, there was a significant positive correlation with Fleischer ring (Table 2). These correlation results imply that Fleischer ring occurred in thinner and more asymmetric corneas of KC relatives. There was a significant positive correlation with prominent corneal nerves for all topographic indices, except for I-S. Significant negative correlation between prominent nerves and central

Table 3 Comparison of groups of individuals in the studied population

Parameters of KC family members (*n*)	Pachymetry	KSI	KISA	3 mm Fourier asymmetry	6 mm Fourier asymmetry	I-S
With Fleischer ring (36)	510.87 (±40.23)	6.20 (±15.94)	261.37 (±1497.80)	0.43 (±0.35)	0.67 (±0.54)	2.48 (±4.91)
Without Fleischer ring (81)	530.17 (±32.90)	2.49 (±9.50)	19.02 (±91.67)	0.30 (±0.23)	0.43 (±0.30)	1.06 (±2.50)
P	0.017	0.010	<0.001	<0.001	<0.001	<0.001
With prominent nerves (17)	506.72 (±48.44)	10.45 (±23.31)	566.07 (±2163.86)	0.50 (±0.43)	0.74 (±0.74)	1.41 (±2.07)
Without prominent nerves (100)	527.96 (±31.79)	2.48 (±8.22)	13.24 (±28.19)	0.31 (±0.24)	0.47 (±0.31)	1.51 (±3.67)
P	0.083	0.006	0.467	0.002	0.008	0.349
With prominent nerves (17)	506.72 (±48.44)	10.45 (±23.31)	15.60 (±2163.86)	0.50 (±0.43)	0.74 (±0.74)	1.41 (±2.07)
Without prominent nerves and without Fleischer ring (70)	530.55 (±31.86)	2.02 (±8.17)	12.90 (±31.79)	0.28 (±0.21)	0.41 (±0.26)	1.03 (±1.50)
P	0.024	0.001	0.907	<0.001	<0.001	0.029

KC family members having corneal Fleischer ring compared to those without Fleischer ring have significantly thinner corneas (*t*-test) and higher values of all topography indices (Mann-Whitney rank sum tests). KC family members with prominent corneal nerves compared to those having no prominent nerves show significantly higher values of KSI, 3 and 6 mm Fourier asymmetry indices (Mann-Whitney rank sum tests). KC family members having prominent corneal nerves compared to those without prominent nerves and without Fleischer ring have significantly thinner corneas (*t*-test) and higher values of all topography indices except for KISA (Mann-Whitney rank sum tests).

corneal pachymetry data indicated that prominent nerves were more likely to appear in thinner corneas (r=-0.235; Table 2).

Central corneal pachymetry values negatively correlated with all examined topographic indices (r<-0.3 for all indices, correlation was the highest for Fourier asymmetry indices 3 and 6 mm r=-0.425, -0.427, respectively).

There was no significant correlation between Fleischer ring and prominent nerves in these corneas, indicating that prominent nerves and Fleischer ring did not frequently occur in the same individuals (Table 2).

In accordance with correlation statistics, siginificant differences were found between relatives having or not having clinical signs of KC, by t-tests. Table 3. shows that relatives with Fleischer ring had significantly thinner corneas and higher values of each videokeratography index compared to those without Fleischer ring. Relatives with prominent corneal nerves displayed higher KSI, 3 and 6 mm Fourier asymmetry indices than those having no prominent nerves (Table 3). Since Fleischer ring and prominent corneal nerves were not frequently concomitant, comparison of corneas with prominent nerves to all other family member corneas including corneas with Fleischer ring- which were thinner and more asymmetrical- yielded significant differences only for KSI and 6 mm Fourier asymmetry. Accordingly, when corneas of relatives with prominent nerves were compared to those who had neither prominent corneal nerves nor Fleischer ring significant differences were found for all parameters except for KISA (Table 3).

Out of 142 control individuals, 1 had only Fleischer ring, 2 had both Fleischer ring and prominent nerves, 2 had only prominent nerves. Correation statistics in this dataset was not reliable because of low number of cases.

Control individuals with Fleischer ring and prominent corneal nerves showed significantly higher values of KSI (P=0.048) and KISA (P=0.012) indices, but were not significanly thinner than controls without Fleischer ring and without prominent nerves (P>0.1).

DISCUSSION

In our dataset of 47 sporadic KC families, occurrence of Fleischer ring and prominent corneal nerves was observed on corneas of unaffected relatives. Of these pathognomic clinical signs, the most remarkable one was the corneal Fleischer ring which was present in almost every third family member (30.8%). This result is similar to the occurrence of Fleischer ring detected in mild KC (27.27% in <45 D keratometry readings) [24]. In manifest KC, we found Fleischer ring in 71.7% of the patients, which is consistent with the results of previous studies of 57% -87% [25,26]. In the healthy control population, Fleischer ring (2.1%), prominent nerves (2.8%) and corneal asymmetry still occurred rarely (Figure 1). In the examined population, Fleischer ring was mostly faint in the corneas of KC family members and controls. To the best of the authors' knowledge, this is the first study of an examination of Fleischer ring and prominent corneal nerves among unaffected relatives of KC patients.

Based on our correlation and t-test results, the presence of Fleischer ring and prominent nerves was associated with features of KC. Family members who exhibited Fleischer ring and prominent nerves had thinner and more asymmetric corneas than those without Fleischer ring and prominent nerves. In control subjects, corneas displaying a Fleischer ring and prominent nerves were more similar to KC corneas (shown by significantly higher KSI and KISA values), but were not thinner than other normal corneas.

Interestingly, prominent nerves and Fleischer ring did not always occur together in the same cornea. It is well known that these signs occur with unequal frequencies in KC [1]. It is believed that KC is a complex disease, both genetic and environmental factors playing a role in its pathogenesis [9]. Fleischer ring is supposed to develop as a consequence of altered response of KC epithelial cells to oxidative stress [27]. Recent corneal *in vivo* confocal microscopy studies consistently have shown that even in mild KC, the subbasal nerve morphology is grossly abnormal [28]. This might be explained by altered regulation of nerve growth factor receptor expression in the KC cornea [29]. These different etiological factors might explain why Fleischer ring and corneal nerves do not always occur together in KC or forme fruste corneas.

It was unexpected to find Fleischer ring in normal controls. Nevertheless, supposing that forme fruste KC is an incompletely developed KC, its occurrence in normal controls is possible. A recent study reported the presence of bilateral Fleischer ring in a subject who had only mild topographic asymmetry, average central corneal thickness, low myopia with stable refraction, normal best corrected visual acuity and normal retinoscopic reflexes. The example of this patient, who was considered as forme fruste KC, indicated that this condition, *i.e.* corneal iron ring can occur without a positive family history and characteristic topographic signs of KC[30].

In our family members, minimal topographic alterations were observed and associated with the presence of Fleischer ring and prominent corneal nerves. One of the examined parameters in this study was central corneal thickness measured by ultrasound pachymetry. Central corneal pachymetry alone is unreliable in the diagnosis of KC, nevertheless, decreased thickness can be a good indicator of similarity to KC and together with the videokeratographic indices this parameter supported that Fleischer ring and prominent nerves tend to occur in corneas more similar to KC. However, the examination of the posterior corneal surface and pachymetry with modern diagnostic methods (*i.e.* Scheimpflug imaging or Orbscan) would be helpful in future studies, since the earliest signs of KC can be more accurately detected with these techniques and correlations will be given further impact with the addition of posterior topographical information[31,32].

In spite of sophisticated imaging techniques, detecting subclinical KC is still challenging in many cases. Identifying subclinical/forme fruste cases, however, is important both for genetic studies and selecting high risk refractive surgery candidates [13,33,34]. Based on our results, it can be suggested that a thorough search for Fleischer ring and prominent nerves in the cornea can help to decide whether or not to diagnose subclinical KC in borderline cases.

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