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Manifest diabetes and keratoconus: A retrospective case-control study

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Abstract *Purpose:* To assess the influence of diabetes on the development of keratoconus to show whether biomechanical effects are also reflected in epidemiology. The two diseases have opposite impact on the biomechanics of the corneal stroma: manifest diabetes stiffens the cornea, whereas keratectasia weakens the tensile strength of the stroma. *Methods:* The retrospective case-control study included files of 1142 patients, with 571 patients in the case group (keratoconus patients) and 571 in the control group (clinical population). The groups were well matched with respect to sex and age. We established the number of diabetics in both groups and compared it statistically by means of the odds ratio to determine whether diabetes can be interpreted as having a „protective effect" whether it is a „risk factor" for the development of keratoconus. *Results:* Two patients of the keratoconus group had manifest diabetes that developed many years after the diagnosis of keratoconus, while nine cases of diabetes were found in the control group. Statistical analysis revealed a strong protective effect of manifest diabetes regarding keratoconus (odds ratio=0.2195, P=0.034). This effect was evident only in type II diabetes patients. *Conclusions:* The protective effect of manifest diabetes may be explained by the induction of cross-links in the stroma, preventing biomechanical weakening of the cornea. This study shows that different biomechanical changes can be superimposed and assume epidemiological relevance.

Introduction

Keratoconus is a vision-threatening condition accompanied by reduced biomechanical strength of corneal tissue. The difference in biomechanical constants between keratoconus corneas and normal corneas has been demonstrated experimentally [1, 3, 10], but the morphological or biochemical cause of this effect is unknown.

The tensile strength of polymers and collagenous tissue can be increased using various techniques [4, 8]. One of these methods of inducing cross-links employs a biochemical reaction cascade called nonenzymatic glycosylation (synonym: glycation) triggered by interstitial glucose [6, 7, 11, 13]. Such cross-links have been detected in connective tissue of diabetic patients and animals [2] and in detail in the cornea [15] but may also occur during aging [7].

The fundamental question of this study was whether cross-links induced during diabetes in the cornea may be powerful enough to compensate for the weakening processes occurring during the development of keratoconus. Because both diseases are relatively rare a case-control study may be the appropriate method for an etiologic investigation [9] testing the hypothesis that diabetes has a protective effect on the cornea regarding the development of keratectasia.

Materials and methods

Study populations

Keratoconus develops mainly after puberty. A potential influence of diabetes on keratoconus must be studied in young patients; therefore, we selected the range of 20–40 years of age for the case and the control group.

The files of 879 consecutive cases of corneal transplantation because of keratectasia between 1990 and 1998 were reviewed in the departments of ophthalmology of the universities of Dresden, Erlangen, and Kiel. The eligibility criteria for potential cases were: (1) the diagnosis of keratoconus had to be substantiated by the history of refraction, clinical diagnostic signs (for example Fleischer ring, corneal tilting, Vogt's striae, or corneal thickness maps), and corneal topography; (2) the history of the patients included clear statements on presence or absence of manifest diabetes, type of diabetes, and onset of diabetes; (3) the age of the patient was between 20 and 40 years at the time of diagnosis of keratoconus. The onset of manifest diabetes was defined as the month and year when the first treatment was started (dietetic or medical). Based on these criteria we found 571 cases for the keratoconus case group.

The control group consisted of 571 consecutive patients referred to our department between 1993 and 1998. The inclusion criteria differed from those for the keratoconus study group in two points: (1) the final diagnoses were known to be not associated with diabetes (congenital cataract, rhegmatogenous retinal detachment, trauma, etc.) and (2) the patients did not demonstrate any clinical sign of keratectasia as defined in the case group. The age of those in the control group was between 20 and 40 years at referral to our clinic. The control group was matched with the study group regarding sex using the χ^2-test for independence and regarding age using the χ^2-similarity test with 20 degrees of freedom (21 age classes).

Statistical analysis

In order to avoid an underpowered study [9], we estimated the minimal sample size of the keratoconus study group necessary to obtain a statistically significant statement on a zero prevalence of manifest diabetes in keratoconus patients. Assuming a prevalence of manifest diabetes in the community of approximately 1% [6], the minimal sample size may be approximated by n=5/1%=500 patients with an error probability of 1% [14].

For statistical comparison of the prevalences of manifest diabetes in the case group and the control group the odds ratio was derived from a 2×2 table (Table 2) and the 95% confidence interval on the estimated odds ratio was calculated [14]. The statistical significance of the exposure (manifest diabetes) on the disease (keratoconus) was determined by the one-tailed χ^2-test of Pearson, Mantel and Haenszel [14] and Fisher's exact test. The criterion of statistical significance was $P<0.05$.

Results

The demographic data on the case group and the control group are listed in Table 1. The difference in age and sex distribution was not statistically significant, indicating a sufficient balancing of the groups regarding these parameters (χ^2_{sex}=1.17; P_{sex}=0.28 and χ^2_{age}=19.67; P_{age}=0.5).

The prevalence of manifest diabetes in the two groups is presented in Table 2. There is a statistically significant association between diabetes and keratoconus. The odds ratio is 0.2195 with a 95% confidence interval ranging from 0.054 to 0.892 (χ^2=4.4939, P=0.034; Fisher, P=0.037). This small odds ratio may be interpreted as showing a strong protective effect of diabetes regarding keratoconus.

Table 1 Demographic parameters of the study populations

	Age (mean±SD)	Sex (M/F)
Case group (keratoconus)	28.86±5.79	399/172
Control group	29.45±5.75	382/189

Table 2 The 2×2 table of cases (keratoconus) and controls with and without exposure to the suspected risk factor (diabetes)

Exposure (diabetes)	Keratoconus group	Control group
Present	2	9
Absent	569	562

Both diabetic patients of the keratoconus group suffered from type I diabetes, whereas in the control group four candidates had diabetes type I and five had diabetes type II. The calculated odds ratio for type I diabetes is 0.4938 (95% confidence interval 0.093 to 1.669, P=0.41, no effect) and for type II diabetes the odds ratio is zero (95% confidence intervall 0.0 to 0.594, P=0.024, strong protective effect).

In both diabetic patients of the keratoconus group the diagnosis of keratoconus (at the ages of 21 and 30 years) was established many years before the development of diabetes (at 39 and 37 years).

Discussion

A previously unreported finding from this study is the significant "protective effect" of manifest diabetes regarding keratoconus, which means that the risk (expressed by the odds ratio) of diabetic patients of developing keratoconus is smaller than that of nondiabetic patients. This statistical conclusion is reinforced by the fact that the two (out of 571) keratoconus patients with manifest diabetes had had keratoconus many years before the diabetes developed. The postulated protective effect appears to be greater in diabetes type II than in unspecified manifest diabetes. However, it was not specified in the patient files how diabetes I was defined, so there is no guarantee that the distinction between types I and II was correct.

A frequent misinterpretation of such a statistical relation is the a priori assumption of a causal relationship. One could speculate that the significant absence of diabetes in keratoconus patients is related to a direct protective

power of a diabetic microenvironment inside the corneal stroma, including the simplest interpretation of glucose or glucose derivates a cross-linking substances in the corneal stroma. On the other hand, statistics may merely indicate the presence of one or several confounders, e.g. a negative genetic association of keratoconus and diabetes, since both diseases are considered partially hereditary in origin [5]. It must also be emphasized that, although statistically significant, the results could be due to chance.

The previously mentioned biomechanical effect of glycosylation may support the hypothesis of the protective effect of diabetes and may explain the fact that in the two cases of diabetes in the keratoconus group, keratoconus preceded diabetes by many years. Covalent cross-links induced by glycosylation have been shown to increase the biomechanical strength of diabetic skin and tail tendons of rats [13]. Also, in human corneas of diabetics the collagen is modified by glycosylation [15]. Based on the current knowledge about the mechanisms of glycosylation, experts believe that it is not the initial glucose adduct but subsequent oxidation products known as advanced glycation endproducts (AGEs) that induce cross-linking not only of collagen but also of elastin [4] and proteoglycans [12], both inter- and intramolecularly. The formation of cross-links within and between collagen fibers in diabetes is consistent with the reduced elasticity of retinal capillaries, renal glomeruli, and arterial vessel walls, the well-known characteristics of diabetic microangiopathy [11]. Experimental cross-linking of corneal stroma by means of glucose demonstrated a significant biomechanical effect after incubation for 2 weeks [16]. Other techniques of cross-linking also increase the tensile strength of the cornea [17]. An alternative explanation of the protective effect may be the resistance of cross-linked collagen to proteolytic degradation, a process that has recently been considered important in the development of keratoconus [18].

Keratoconus usually starts in puberty and is typically diagnosed after 20 years of age because of progressive myopia and astigmatism. The process of weakening of the cornea may cease later, leaving the cornea in the state of "*forme fruste* of keratoconus". The key finding of this investigation, that keratoconus may not develop after manifest diabetes, may indicate that diabetes can induce such a process to reverse or can compensate the biomechanical weakening of the cornea. Clearly, the most appropriate range of age to investigate this question would have been the decade between 10 and 20 years. However, at that age the prevalence of diabetes and keratoconus is so small that, at a rough estimate, at least 2400 files would have had to be reviewed, far more than we had access to. Even for the decade of 20 to 30 years of age the number of files required would have been in the order of 1000.

The most critical point of a case-control study is the selection criteria for the control group, including the definition of the diseases. In this study, only patients being treated for diabetes are considered diabetic. It is certainly possible that some of the patients in both groups either had diabetes prior to the diagnosis being made or had undiagnosed, and therefore untreated, diabetes. However, the probability of such undiagnosed diabetes can be assumed to be identical in the case and the control group.

There was no significant difference between the two groups in age and sex, indicating successful matching of the two groups. However, the prevalence of 1.5% of manifest diabetes in the control group is of concern because the prevalence of manifest diabetes in the age group investigated in Germany is reported to be 1.0% and less (P. Heinke, Institut für Diabetes, Karlburg, Germany, unpublished data). Although patient files with diagnoses known to be associated with diabetes were carefully excluded from the control group, we cannot totally rule out the possibility that some of the diagnoses, such as uveitis and retinal degenerations in myopia, are weakly correlated with manifest diabetes. On the other hand, exclusion of diabetic-related diseases from the control group should lead to underestimation of the prevalence of diabetes and the protective effect rather than overestimation.

In summary, the results of this study suggest that manifest diabetes provides a statistically significant "protective effect" regarding the development of keratoconus. While we are confident in the relevance of this statement, this study is nevertheless retrospective, and a prospective cohort study can provide stronger evidence of a causal relationship between juvenile diabetes and keratectasia. This investigation shows that biomechanical effects regarding cross-linking are also reflected in epidemiological results.

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