ABSTRACT

Objective: To assess the main clinical, genetic, histopathological and ultrastructural features of Mexican patients with macular corneal dystrophy, and to compare the results with those previously reported.

Method: We analyzed six cases where a histopathologic diagnosis of macular corneal dystrophy had been made between 1957 and 2004.

Results: Clinically, all corneas showed focal grayish-white stromal opacities with diffuse edges. Histopathologically, intrastromal granules stained strongly positive with Alcian blue and colloidal iron. Transmission electron microscopy showed enlargement of smooth endoplasmic reticulum and the presence of intracytoplasmic vacuoles that corresponded to glycosaminoglycans. Genetic analysis showed novel mutations in the CHST6 gene in 2 of the patients.

Conclusions: Females were more affected than males and the mean age at the time of diagnosis was older than that reported previously, however the clinical, histopathological and ultrastructural features were similar to those of previous reports. As described.

RESUMEN

Objetivo: Determinar las principales características clínicas, histopatológicas y ultraestructurales en pacientes mexicanos con distrofia macular de la córnea, así como las alteraciones genéticas en algunos de ellos y compararlas con lo informado en la literatura.

Método: Se recopilaron un total de seis casos con diagnóstico histopatológico de distrofia macular corneal registrados de 1957 a 2004.

Resultados: Clínicamente todas las córneas presentaban opacidades estromales blanco grisáceas de bordes difusos. Histopatológicamente se demostró la presencia de depósitos finamente granulares entre las laminillas del estroma corneal, que resultaron intensamente positivos con las tinciones de azul alciano e hierro coloidal. Por microscopía electrónica de transmisión se demostró dilatación del retículo endoplasmático liso y la presencia de vacuolas intracitoplásmicas con un material electrodenso, correspondiente a glicosaminoglicanos. El análisis genético en dos de los pacientes demostró mutaciones en el gen CHST6.
INTRODUCTION

Cornea Macular Dystrophy (CMD) is a recessive autosomic disorder which exhibits alterations in the metabolism of keratan sulphate. It is the least frequent stromal dystrophy but which expresses at the earliest age. This dystrophy is not associated to systemic abnormalities. However, there is a case report of a patient with this disorder associated to hypotrichosis (1).

The literature has described two types which are undistinguishable both clinically as well as histologically. Type 1 is characterized by lack of sulphated keratan sulphate in the cornea, serum and cartilage, whereas Type 2 exhibits sulphated keratan sulphate in the same locations but with a synthesis of 30% below normal values.

The clinical condition of these patients consists in a clear cornea at birth which becomes gradually opaque after age 3. The presence of white-grayish local stromal opacities can be observed which, in the first stages of the disease, are superficial and central but gradually extend to the deep stroma and to the periphery of the cornea. The endothelium may be affected as well.

The main symptoms of this dystrophy comprise reduction of visual acuity and photophobia.

Histopathologically, the disease is characterized by the presence of extra-cellular deposits of acid mucous substances which correspond to glicosaminoglicanes (GAG), both between stromal lamellae and between the cytoplasm of endothelial cells. These deposits are intensely positive with alcyan blue and colloidal iron tincture. By means of ultrastructure a dilatation of the smooth endoplasmic reticule is observed together with the formation of intracytoplasmatic vacuoles due to the presence of an electrically dense material which corresponds to GAG.

The literature describes mutations at the level of chromosome 16q22, in a gene called CHST6 which encodes the corneal enzyme glucosamine N-acetyl-6 sulphotransferase (C-GlcNac-6-ST), which operates as a carbohydrate sulphotransferase (2). The treatment for these patients in advanced stages of the disease consists in penetrating keratoplasty (3).

This work has the main objective of determining the main clinical, histopathological and ultra-structural characteristics of Mexican patients with CMD as well as the genetic alterations in some of them, and compare these with those reported in the literature.

SUBJECTS, MATERIAL AND METHODS

We reviewed all the cases with histopathological CMD diagnostic recorded in the period between 1957 and 2004. Of all cases, we made 5-micron sections of tissue in 10% formol and included in paraffin, which were dyed with hematoxiline and eosin, Schiff per iodine acid, alcyan blue and colloidal iron.

In one of the cases we also carried out semi-fine sections, dyed them with tolouidine blue and selected determined specific areas for study with electronic transmission microscope by means of the conventional technique.

In two patients, we took 30 ml of peripheral venous blood and of 10 ml of each the DNA was extracted by means of an isolation kit. The DNA extracted together with the remaining 20 ml of fresh blood of each patient were sent to the Medical Center of Duke University, Durham, North Carolina.
where one of the authors (GKK) carried out the genetic analysis together with his team. The blood sample was taken after each of the participating patients signed an informed consent document.

RESULTS

The main clinical findings are summarized in Table I. The data comprise six patients, four women and two men, with age intervals between 24 and 41 and an age average at the time of the diagnostic of 32.5 years.

In all case, the involvement was bilateral and expressed clinically in a very similar way, characterized by the presence of corneal opacities of 0.5-1 mm diameter, grayish white and diffuse edges, involving the entire stromal thickness, predominantly the superficial third, as well as the endothelium. The stroma localized between the opacities exhibited a diffused veiling (fig. 1). The evolution time was long in half of the cases, and ranged between 5 and 14 years. In the other half, the evolution was not specified. The clinical diagnostic was macular dystrophy in four patients, granular dystrophy in one and familial sub-epithelial amyloidosis in the remaining patient.

The treatment of choice in all patients was penetrating keratoplasty, in four unilateral and bilateral in two. None of the patients exhibited relapse in the follow-up period, which ranged between 3 and 9 years after the corneal transplant.

Histopathologically, all the corneas exhibited deposits of a fine granular material between the layers of the corneal stroma, predominantly in the superficial third.

Said deposits were present in the cytoplasm of endothelial cells. They were intensely positive with alcyan blue and colloidal iron tincture (fig. 2). No immune tinctures were made in any case due to the absence of antibodies for proteoglycans and glycosaminoglycans. Ultra-structure revealed dilatation of the smooth endoplasmic reticule and the presence of intra-cytoplasmatic vacuoles with electrically dense material corresponding to GAG (fig. 3).

In the genetic analysis, two mutations were found in one patient while in another only one mutation was found (table II). No genetic alterations were found in other regions besides the CHST6 gene.

Table I. Clinical findings in patients with CMD

<table>
<thead>
<tr>
<th>#</th>
<th>Age/Sex</th>
<th>Eye</th>
<th>Evol. time</th>
<th>Clinical diagnostic</th>
<th>Treatment</th>
<th>Follow-up (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30/F</td>
<td>BE</td>
<td>NS</td>
<td>Mac. Dystrophy</td>
<td>PKP, BET</td>
<td>(9) NR</td>
</tr>
<tr>
<td>2</td>
<td>32/F</td>
<td>BE</td>
<td>12 years</td>
<td>Mac. Dystrophy</td>
<td>PKP, BET</td>
<td>(9) NR</td>
</tr>
<tr>
<td>3</td>
<td>34/F</td>
<td>BE</td>
<td>14 years</td>
<td>Mac. Dystrophy</td>
<td>PKP, LE</td>
<td>(5) NR</td>
</tr>
<tr>
<td>4</td>
<td>24/M</td>
<td>BE</td>
<td>NS</td>
<td>Granular Dyst.</td>
<td>PKP, LE</td>
<td>(4) NR</td>
</tr>
<tr>
<td>5</td>
<td>41/M</td>
<td>BE</td>
<td>NS</td>
<td>Amyloidosis</td>
<td>PKP, RE</td>
<td>(3) NR</td>
</tr>
<tr>
<td>6</td>
<td>34/M</td>
<td>BE</td>
<td>5 years</td>
<td>Mac. Dystrophy</td>
<td>PKP, LE</td>
<td>(3) NR</td>
</tr>
</tbody>
</table>

BE = Both Eyes; NS = Not Specified; PKP = Penetrating Keratoplasty; LE = Left Eye; RE = Right Eye; NR = No Relapse.
DISCUSSION

In our series of CMD cases, we found a prevalence of the female gender, in contrast with reported in other series in which no gender-based prevalence was found.

The average age of our patients at diagnostic time was of 32.5 years, greater than the mean age reported in the literature. We believe that the explanation may be that the patients of our series came from rural communities far from big cities and therefore visited the practice in more advanced stages of the disease, when it became symptomatic and caused a reduction in visual acuity.

Due to the fact that this dystrophy is inherited with a recessive autosomic transmission pattern, each affected individual can be expected to have two mutation in the CHST6 gene. Of the two patients we studied, one had a single mutation of this gene. Although the de novo mutations and the deletion we found have not been reported previously, they were located in the CHST6 gene region. In the study by Akama et al (2) they found several mutations as well as deletions and/or long substitutions in the region which encodes for the CHST6 gene. A further study by El-Ashry et al (4) identified 6 de novo mutations in 5 different families. Furthermore, in a study by Aldave et al (5) four different mutations were found in the same gene. The results of these studies, to which our study is added, demonstrate that the genetic alterations which can be found in the region which encodes for the CHST6 gene can be varied: either de novo mutations (homozygous or heterozygous), deletions and/or substitutions. However, to date the clinical and histopathological characteristics of CMD patients undistinguishable regardless of their genetic substrate. A number of reports in the literature describe the presence of homozygous in this disease, probably due to the technical difficulty involved in finding both mutations (4).

Akama et al (2) analyzed the messenger RNA in human corneas and proved that the normal expression of this gene is correlated to the presence of sulphated keratan sulphate in the cornea, a normal characteristic which enhances the laminar organization and therefore corneal transparency. On the other hand, Hasegawa et al (6) demonstrated that a reduction in the activity of C-GlcNac-6-ST (secondary to an abnormal expression of the CHST6 gene) is related to the presence of non-sulphated keratan sulphate in human corneas, and the presence thereof caused corneal opacities. In summary, said genetic abnormalities are responsible for losses in the function of sulphotransferase in carbohydrates, an alteration which is responsible for the phenotype of cornea macular dystrophy, secondary to the presence of non-sulphated keratan sulphate, which entails a loss in the organization and normal structure of the corneal stroma substance.

Even though the literature reports relapse of dystrophy in grafts, the rate thereof is the lowest compared to other stromal dystrophies (7-10). None of our cases exhibited relapse in a follow-up period of up to 9 years after the corneal transplant. Kuchle et al (7) reported a case with Type 2 CMD relapse 49 years after a penetrating keratoplasty.

![Image: Electromicrography showing large intra-cytoplasmatic vacuoles occupied by electrically dense material (glycosaminoglycans).](image)

Table II. Genetic alterations in CMD patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Change of nucleotides</th>
<th>Change of aminoacids</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G888T</td>
<td>val66phe</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>2</td>
<td>nts 1293 a 1313 G1693A</td>
<td>deletion of aa 201 al 207 arg534cys</td>
<td>Homozygous Heterozygous</td>
</tr>
</tbody>
</table>
To the extent of our knowledge, this is the first series of cases of Mexican CMD patients reported in Latin America, with a greater prevalence in women, with a higher age average and without relapse of the disease in some cases up to 9 years after penetrating keratoplasty. The clinical presentation, the histopathological and ultra-structural findings, as well as the genetic alterations are very similar to those previously reported in other series.

REFERENCES