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1-Year Follow-up Study of Endothelial Cell Density Loss after Penetrating Keratoplasty

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A B S T R A C T

High endothelial cell densitiy (ECD) is essential for the corneal graft clarity. We evaluated ECD loss in 120 eyes that underwent penetrating keratoplasty (PK) in Eye Clinic Svjetlost in a one year follow up period. Patients were divided into 3 groups of high (N=35), intermediate (N=31) and low risk (N=54) for graft failure. Postoperative central endothelial density, coefficient of variation in cell area (polymegathism), percentage of hexagonal cells (pleomorphism) in comparison to preoperative donor cell measurements were determined in the following postoperative time-points of 1, 2, 3, 6, 9 and 12 months. There were no significant differences in the preoperative ECD values, storage time, donor age or surgical procedures between groups. Throughout all time points intermediate group had the greatest statistically significant ECD loss as compared to high and low risk groups. There were no significant differences between high and low risk group. After 12 month post PK, intermediate risk group had 28.38% ECD loss as compared to 24.07% in high and 23.03% ECD loss in low risk group. Coefficient of variation in cell area (CV) was for high risk group 0.34, intermediate 0.40 and low risk 0.31 which was not significantly different between groups. Percentage of plemorphism in high risk was 54%, intermediate 58% and in low risk 48% which was significantly different as compared to other two groups. Our study showed that corneal pathology is among others, very important prognostic factor for ECD after PK. However, longer follow up period is needed.

Abbreviations: ECD – Endothelial cells density, PK – penetrating keratoplasty, CV – Coefficient of variation in cell area

***Key words**: Endothelial cells, corneal graft*

Introduction

Corneal endothelial cells (EC) are the monolayer of hexagonal unreplicated cells that lines posterior corneal surface and are derived from the neural crest during embryologic development[1]. The principal physiological function is to provide stromal deturgenscense by allowing leakage of solutes and nutrients from the aqueous humor to the more superficial layers of the cornea while at the same time actively pumping water in the opposite direction, from the stroma to the aqueous. Therefore, high endothelial cell density (ECD) is essential for corneal clarity and therefore good visual clarity[2].

The loss of endothelial cells is a natural process of aging as well the changes in morphology. Human central ECD decreases at an average 0.6% per year in normal corneas during life with gradual increases in polymegathism and pleomorphism[3]. Some of the factors that can influence ECD decrease are surgical trauma, methods and durations of corneal storage, exchange between donor and recipient cells, cell aging and immune reactions as well as diagnosis like preoperative diabetes and glaucoma[4,5,6].

All surgical treatments of anterior segment that involve the entry into the anterior chamber can cause the damage of the endothelial cells. Primary, the cataract surgery as well the corneal transplantation are two main surgical procedures that can cause ECD loss and corneal decompensation[6,7,8]. Improved storage techniques can also affect survival of EC cells and therefore provide lower rate of late endothelial failure, such as corneal preservation methods which enable donor cornea to pass the »stress-test« during storage instead in recipients eye[9,10,11]. Therefore, those corneas with ECD loss of 20% or more are not grafted[10]. The preoperative EC number is a very important factor that influences the graft sur-

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vival rate although the studies on this issue are conversely[11,12].

In this study, we wanted to investigate how and whether the preoperative corneal pathology in the recipient eye (high, intermediate and low risk) influences on ECD loss after penetrating keratoplasty.

Materials and Methods

We prospectively evaluated 120 eyes that underwent penetrating keratoplasty (PK) in Eye Clinic Svjetlost in a one year follow up period. Patients were divided into 3 groups of high (N=35), intermediate (N=31) and low risk (N=54) for graft failure. The group of high risk diagnosis included herpetic keratitis (N=9), corneal combustion (N=5), rejected graft (N=11), Stevens- Johnson syndrome (N=2), postraumatic vascularised leucoma (N=6) and postinfective keratitis (N=2). The group of intermediate risk included bullous keratopathy (N=14), keratoglobus (N=4) and PK surgery on the other eye (N=13). Finally, the low risk group included keratoconus (N=45), Groneuw corneal dystrophy (N=7), and Reis Bucklers corneal dystrophy (N=2). We evaluated and calculated postoperative central endothelial density in comparison to preoperative donor cell measurements, coefficient of variation in cell area (polymegethism), percentage of hexagonal cells (pleomorphism) (CSO Specular Microscope, Italy) and corneal thickness with non-contact optical coherence tomography customized for the anterior segment (Zeiss Visante™ OCT, Germany) in the following postoperative time-points of 1, 2, 3, 6, 9 and 12 months.

All surgeries were done by the same surgeon and by the same surgical technique of PK. Donor corneal tissues were obtained from the certificated eye banks from USA (International Sight Restoration) and Europe (Všeobecna Fakultni Nemocnice, Prague; Salhgrenska University Hospital, Sveden). They were carefully selected under central corneal density criteria higher than 2300 cells/mm^2 and age correlation between donor and recipients not greater than 20 years. Only patients without any sign of corneal graft reaction in the first postoperative year were included in this study.

Results

Mean preoperative ECC for the group of high risk was 2825.81 cells/mm^2, intermediate of 2946.44 and low of 2929.52 cells/mm^2. There were no significant differences in the preoperative ECC values between groups. 1 month following procedure, ECC loss in high risk group was 13.06%, intermediate 17.34% and low risk of 14.75%, following trend after 3 month of 17.07% in the high risk group, 25.16% in intermediate and 20.94% in low risk group. At month 3, there was statistical significance between intermediate group values and high and low risk group ($p<0.05$, student T-test). 6 month following PK, ECC loss was in high risk 17.48%, similar to month 3, but in climbing trend of 26.66% of ECC loss in intermediate group and of 21,88% in low risk group, which was significantly different between all 3 groups ($p<0.05$, student T-test). The trend continued through the month 9 where it significantly increased in high risk group (20.90% ECC loss), and remained stable in other two groups (intermediate of 27.77% and low risk of 21.83%). 12 months postoperatively, final results were 24.07% of ECC loss in high risk group, 28.38% in intermediate and 23.03% in low risk group. Significant difference was in intermediate group versus high and low risk groups ($p<0.05$, student t-test). There was no significant difference between high and low risk group. Graph 1 is showing ECC trend loss through time points in all three groups. Coefficient of variation in cell area (CV) was for high risk group 0.34, intermediate 0.40 and low risk 0.31 which was not significantly different between groups. Percentage of hexagonal pleomorphism in high risk was 54%, intermediate 58% and in low risk 48% which was significantly different as compared to higher risk groups ($p<0.05$, student t-test).

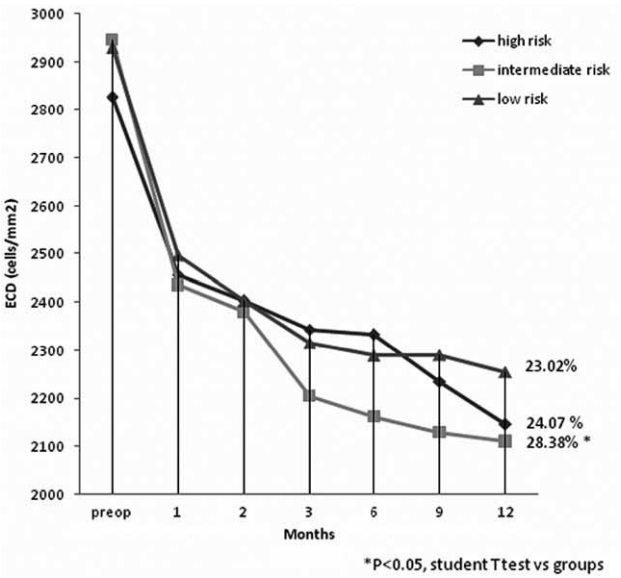

*Fig. 1. Endothelial cell density loss throughout time between high, intermediate and low risk groups. (*p<0.05, student t-test).*

Discussion

High endothelial cell count is of the great importance for the clarity and stability of the corneal graft. There are several risk factors affecting endothelial cell density that can be influenced on, such as method of donor cornea preservation, storage time and surgical procedures. There is not strong evidence that human corneal cells can divide in vivo, although several studies showed that at least some endothelial cells are capable of dividing and proliferating in humans as well as in experiments preformed on rats following PK, what proves existing of in-vivo re-endothelialization[13]. However, it is well known that endothelial cells can divide in cultured corneas. To-

day, there are different approaches that can efficient the survival of endothelial cells such as the use of growth factors and molecules that inhibit apoptosis in preservative medium[14,15]. Studies that investigate the influence of donor tissue storage time and donor endothelial survival, found no significant correlation between the characteristic of storage time and decline of ECD[11]. Advanced surgical techniques of anterior segment of the eye can contribute to lower rate of EC loss. Several studies investigated the influence of technique in cataract surgery as well as different approaches to corneal transplantation on graft survival and endothelial outcomes after DSEK(Descemet stripping endothelial keratoplasty) and DSEAK (Descemet stripping automated endothelial keratoplasty)[8,16,17] and did not show the statistical significant difference in cell loss between these techniques over 1-year postoperative period. In case of »triple« procedure or the anterior positioning of IOL (eg. Verisyse iris claw lens) the ECD loss is higher than in PK alone[18]. Moreover, if the inflammation is present in the post PK period like in highly vascularized eyes, inflammation may also cause an increased ECD loss[19]. Size and site of the incision in phaco surgery as well as in corneal transplantation can also influence on the endothelial cell loss[6–8].

In our study we compared post PK endothelial cell loss between different corneal pathologies divided from the greatest to the lowest graft failure risk group. There was no significant difference in preoperative ECD, storage time, donor age or surgical procedures between groups.

One month postoperatively all groups showed similar decreasing trend of ECC, with intermediate group as the leading one, although there was no significant difference between groups. In one month period difficulty in ECD measurement is very common because of the corneal edema and if measured most of the cells in that period are lost because of intraoperative manipulation. EC loss trend remained the same throughout the whole period of 12 month and was significantly different in intermediate as compared to high and low risk group. On contrary high and low risk group had no significant difference. Intermediate group had the highest endothelial cell density loss of 28.38% because the corneal diseases in the intermediate group such as bullous keratopathy and keratoglobus have primary endothelial cell layer dysfunction. In conclusion our study showed that corneal pathology is also very important prognostic factor in endothelial cell density after PK. However, longer follow up period is needed.

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GUBITAK ENDOTELNIH STANICA ROžNICE NAKON PERFORATIVNE KERATOPLASTIKE: JEDNOGODIŠNJE PRAĆENJE

SAŽETAK

Visoka gustoća endotelnih stanica je ključna za bistroću kornealnog transplantata. U Klinici za oftalmologiju Svjetlost u vremenskom periodu od 1 godine, promatrali smo pad endotelnih stanica kod 120 operiranih očiju metodom perforativne keratoplastike. Pacijenti su bili podijeljeni u 3 skupine visokog rizika (N=35), srednjeg (N=31) i niskog rizika (N=54) za odbacivanje kornealnog transplantata. U vremenskom periodu od 1,2,3,6,9 i 12 mjeseci pratili smo postoperativnu gustoću endotelnih stanica, koeficijent varijacije stanica (polimegatizam), te postotak heksagonalnih stanica (pleoformizam). Preopertaivno između grupa nije bilo statički značajne razlike u vrijednostima broja endotelnih stanica, vremenu prezervacije ili kirurških postupaka. Kroz sve promatrane vremenske intervale, grupa srednjeg

rizika je imala statički značajno najveći gubitak broja endotelnih stanica u usporedbi s grupom visokog i niskog rizika. Između grupe visokog i niskog rizika nije bilo statički značajne razlike. 12 mjeseci nakon perforativne keratoplastike, grupa srednjeg rizika imala je pad endotelnih stanica od 28,38% u usporedbi s gubitkom od 24,07% u grupi visokog rizika te 23,03% u grupi niskog rizika. Koeficijent varijacije stanica je u grupi visokog ruzika bio 0,34, u grupi srednjeg rizika 0,40 te 0,31 u grupi niskog rizika. Postotak pleomorfizma je u grupi visokog rizika bio 54%, srednjeg 58%, te statički značajno niži 48% u grupi niskog rizika. Ova studija je pokazala da gubitak endotelnih stanica nakon perforativne keratoplastike između ostalog ovisi o bolestima rožnice primatelja, međutim potreban je duži period praćenja.