Analysis of Tear Fluid Cystatin C as Biomarkers in Patients with Primary Ocular Tumors

Ayu Aliyah, Rodiah R Lubis, Masitha Dewi S
Ophthalmology Department, Faculty of Medicine, University of Sumatera Utara, Indonesia

Abstract

Introduction: Cystatin C is known to play a role in tumor development and is expected to be used as a biomarker in eye tumors.

Purpose: To analyze levels of tear fluid cystatin C as biomarkers of primary ocular tumors.

Materials and Methods: This study was an analytical observational study with a case-control design. A total of 25 subjects with eye tumors in the study group and 25 subjects without eye tumors in the control group were included. Sampling was conducted from April 2022-July 2022 at the Eye Polyclinic of the Reconstructive Ocuplasty and Oncology division. The Mann-Whitney test was used with a significance level of 5%.

Results: There was a significant difference in the tear fluid Cystatin C level between subjects with eye tumors and without eye tumors ($p<0.001$), with the mean tear fluid Cystatin C in the eye tumor group of 1.43 mg/dL. However, there was a significant difference in the level of tear fluid Cystatin C between the groups of subjects with malignant eye tumors and benign eye tumors ($p=0.042$).

Conclusion: There was a significant relationship between tear fluid cystatin C levels and eye tumors ($p < 0.001$).

Keywords: Benign Ocular Tumor, Malignant Ocular Tumor, Cystatin C, Tear Fluid

Introduction

Epidemiologically, cases of eye cancer are so rare that little information has been reported about the prevalence of eye cancer in the world. In the United States, according to the American Cancer Society, the incidence of eye tumors (new cases) in 2020 was 3,320 new tumor cases. Of these cases, 1,750 cases were men, and 1,570 cases were women. The incidence (new cases) was 1 per 100,000 population, and the prevalence (total cases) is around 12 per 100,000 population. According to Cancer Research UK data, it was reported that around 5,800 people were diagnosed with eye cancer in the UK from 1991-2010. And in 2015, there were 738 new cases, with an average increase of 14% per year (Soebagjo, 2019; ACS, 2021). Cases of eye tumors, in general, were also rarely reported in Asia. In the study by Jung SK et al., it was reported that the incidence of palpebral tumors increased from 0.27 per 100,000 population in 1999 to 0.61 in 2016, with a range of 4.94% increase in cases per year (Guo et al., 2018). Likewise, the incidence of eye tumors in Indonesia was rarely reported specifically and is limited to regions in Indonesia. According to a study by Lubis et al., in 2021, 30 cases of eye tumors were reported in Medan, with a distribution of 17 males and 13 females (Soebagjo, 2019).

Cystatin is a biomarker belongs to the group of endogenous cysteine protease inhibitors that can meet the criteria as a marker of glomerular filtration rate (Maheshwari & Finger, 2018). It is essential in clinical and experimental treatment. The primary purpose of Cystatin is to inhibit the cysteine protease enzyme, as well as to promote cell proliferation, migration, and differentiation. Cystatin C can affect protein catabolism, the development of cancer, bone resorption, hormone control, and modulation of inflammation. Cystatin C is present in patient's tear fluid and serum. All cell types generate Cystatin C at a constant rate. Cystatin C

is a protein that inhibits all cysteine proteases in eye fluid (which is still understudied in comparison to other biological fluids) (Dikovskaya et al., 2013).

A study by Terpos et al. described cystatin C as a multiple myeloma prognostic factor. In addition, Decock et al. study explained that Cystatin C could also have a role in diagnosing the early stages of breast cancer (Terpos et al., 2009; Decock et al., 2008). Dikovskaya et al. (discovered that cystatin C levels rose in both malignant and benign ocular cancers. Based on the aforementioned explanation, it is known that cystatin C can be used as a biomarker of eye tumors (Dikovskaya et al., 2013). To determine the relationship between Cystatin C and primary ocular malignancy, however, additional research is required. As a result, the author aimed to analyze the amounts of Cystatin C in serum and tear fluid as biomarkers of primary eye tumors.

Materials and Methods

This research was conducted with a case-control analytic observational study design among individuals with and without eye tumor with consecutive sampling at the Eye Polyclinic in Universitas Sumatera Utara Hospital and Network Hospital from April 2022 to July 2022. All research subjects were asked to provide informed consent before. This research proposal was approved by the ethics committee of USU medicine.

Measurement of Cystatin C Tear Fluid Levels

Examination of cystatin C levels in tear fluid was carried out by taking 10 microliters of tear fluid in the patient and control samples. The Cystatin C levels in tear fluid was determined using the Cystatin C sandwich enzyme-linked immunosorbent assay (ELISA) kit.

Statistical Analysis

Using a Mann-Whitney test with a 5% significance level, the difference of tear fluid cystatin C in ocular tumor patients and controls was determined.

Results

Demographic Characteristics of Research Subjects

This study included 25 patients with eye tumors and 25 patients without eye tumors. Table 1 shows the demographic characteristics of the research subjects.

Table 1. Demographic Characteristics of Research Subjects

Demographic Characteristics	Eye Tumor (+) (n=25)	Eye Tumor (-) (n=25)
Gender, n (%)		
Man	9 (36)	9 (36)
Woman	16 (64)	16 (64)
Age, years		
Average (SD)	47.92 (17.79)	49.16 (18.05)
Median (Min – Max)	52 (18 – 73)	51 (18 – 76)
Tumor Type		
Malignant Tumor		
Basal Cell Carcinoma	5 (55.6)	-
Rhabdomyosarcoma	1 (11,1)	-
Squamous Cell Carcinoma	3 (33.3)	-
Benign Tumor		
Cavernous sinus hemangioma	1 (6.2)	-

Epidermoid cyst	1 (6.2)	-
Lacrimal Gland Cyst	2 (12.5)	-
Conjunctival Cyst	1 (6.2)	-
Palpebral cyst	2 (12.5)	-
Lipoma	2 (12.5)	-
Nevus	4 (25)	-
Pseudotumor	3 (18.8)	-

Differences in Levels of Cystatin C Serum and Cystatin C Tear Fluid between Subject Groups with and without Eye Tumors

Table 2 displays the Cystatin C levels in tear fluid of individuals with and without eye tumor.

Table 2. Differences in Levels of Cystatin C Serum and Cystatin C Tears Fluid between Subject Groups with and without Eye Tumors

	Eye Tumor (+) (n=25)	Eye Tumor (-) (n=25)	p
Tear Fluid Cystatin C, mg/dL			
Average (SD)	1.43 (1.36)	0.61 (0.23)	<0.001[a]
Median (Min – Max)	1.21 (0.34 – 7.7)	0.59 (0.19 – 1.03)	

Note: [a]Mann-Whitney

The average value of tear fluid Cystatin C in the group of subjects with eye tumors was 1.43 mg/dL. Meanwhile, the subjects without eye tumors had a lower mean of 0.61 mg/dL. Furthermore, the Mann-Whitney test showed a significant difference of Cystatin C levels in tear fluid between groups of subjects with and without eye tumors ($p < 0.001$).

Differences in Tear Fluid Cystatin C Levels Based on Tumor Type
Cystatin C tear fluid levels are displayed in Table 3 for a group of individuals with malignant eye tumors and a group of individuals with benign eye tumors.

Table 3. Differences in Cystatin C Serum and Tear Fluid Cystatin C Levels Based on Tumor Type

	Malignant tumor (n=9)	Benign tumor (n=16)	p
Tear Fluid Cystatin C, mg/dL			
Average (SD)	2.07 (2.14)	1.08 (0.38)	0.042[a]
Median (Min – Max)	1.32 (0.81 – 7.7)	1.09 (0.34 – 1.68)	

Note: [a]Mann-Whitney

The average tear fluid Cystatin C in the group of subjects with malignant tumors was 2.07 mg/dL; meanwhile, the mean in the group of subjects with benign eye tumors was lower at 1.08 mg/dL. In addition, the Mann-Whitney test showed a significant difference in the levels of tear fluid cystatin C between groups of subjects with malignant eye tumors and benign eye tumors ($p = 0.042$).

Discussion

In this study, the majority of subjects in the case group were female, amounting to 16 people (53.3%) as in the control group, with the majority females and the same percentage. This result followed a study by Zloto et al where the annual incidence of uveal melanoma was lower in male than female patients (Zloto, Pe'er, & Frenkel, 2013). However, contrary to the data shown by the American Cancer Society eye cancer in 2022, there were about 3,360 new cases of cancer (mainly melanoma) of the eye and orbit where the majority of patients were male (1,790 in men and 1,570 in women) (ACS, 2021). Another study showed that men over the age of 55 had the highest risk of developing eye tumors (male-to-female ratio: 1.28:1) (Al-Mujaini et al., 2021). This inconsistency among population-based studies may be influenced by different criteria and populations.

Several theories have been proposed to explain gender differences in ocular tumor genesis and progression. In research demonstrating male predominance in the development of eye tumors, it was hypothesized that environmental factors (increasing pollution, exposure to ultraviolet [UV] radiation, and lifestyle behaviors including tobacco and alcohol intake) also influence the incidence of eye tumors (Al-Mujaini et al., 2021). We speculate that because both men and women currently participate in outdoor activities and have similar lifestyles, it is suspected that it may have contributed to the dominance of women in our study.

The mean age in this study in the subjects with eye tumors group was 47.92 years, with the youngest at 18 years old and the oldest at 73 years old. This result was in concordance with the research of Al-Mujaini et al., who found that fifty percent of their patients (n = 123) were above the age of 55. Nonetheless, the analysis revealed a bimodal distribution of patients by age group, with peaks at 15 and 55 years (Al-Mujaini et al., 2021). According to the study by Li et al., there were two peaks in the incidence of malignant ocular tumors, one occurring between the ages of 0-9 and the other between 60-69 (Li et al., 2022). Cancer Research UK stated that older age is a significant risk factor for cancer. It primarily reflects the damage to the cell's DNA that accumulates over time from biological processes or exposure to risk factors. In addition, earlier and greater accumulation of mutations and/or alteration of the tissue landscape due to smoking or sun exposure would lead to a higher incidence of tumor growth risk (Laconi, Marongiu, & DeGregori, 2020).

In this study, the most prevalent malignant tumor type was basal cell carcinoma (BCC), affecting 5 individuals (55.6%). In Western nations, China, Korea, Singapore, Taiwan, and Thailand, BCC is the most prevalent type of malignant eyelid tumor. However, in the Indian population studied by Kaliki et al., squamous cell carcinoma (SGC) was the most prevalent malignant eyelid tumor (Kaliki et al., 2019). BCCs arise from the basal cells of the epidermis and are characterized by a pearly border and pink color with the tendency to ulcerate and bleed. The risk of basal cell carcinoma increase with prolonged sun exposure during the first 2 decades of life and smoking history (Shi, Jia, & Fan, 2019).

In the group of subjects with benign tumors, the most common type of tumor was nevus, totaling 4 people (25%). This result followed the study by Li et al. that found the three most common benign eyelid tumors were nevus, squamous cell papilloma, and cyst, which occurred in the age range of 0-90 years, and most were diagnosed at the age of 30-69 years (Li et al., 2022). Nevus/Nevi is an increased collection of melanocytes, most commonly seen in the skin but can occur in the iris, ciliary body, and choroid. Young age (less than 40 years), inferior location, irregular edges, large feeder vessels, diffuse distribution, seeding, and nodular growth increase the risk for malignant transformation (Marr, 2019).

The group of individuals with eye tumors had a mean tear fluid Cystatin C concentration of 1.43 mg/dL (range: 0.34-7.7 mg/dL), whereas the group of individuals without eye tumors had a mean tear fluid Cystatin C concentration of 0.61 mg/dL (range:

0.19-1.03 mg/dL), with a statistically significant difference (p<0.001). This result was consistent with the findings of Dikovskaya et al., who assessed tear fluid Cystatin C levels in individuals with uveal melanoma to those of control group, wherein serum Cystatin C levels of uveal melanoma patients were significantly greater than those of controls (p<0.01) (Onopiuk, Tokarzewicz, & Gorodkiewicz, 2015; Dikovskaya et al., 2021). Similar results were found in a similar study evaluating a malignant ocular tumor (choroidal melanoma), compared with healthy controls, where tear fluid cystatin C levels were elevated in both eyes, with a significant difference compared to controls (p<0.001) (Dikovskaya et al. 2013). Another study found lower tear fluid Cystatin C concentrations (0.05 ± 0.0003) in healthy individuals than in patients with pterygium (0.13 ± 0.02), with a statistically significant difference (p<0.0002) (Barba-Gallardo et al., 2013).

The average tear fluid Cystatin C in the group of subjects with malignant tumors was 2.07 mg/dL (0.81-7.7 mg/dL), while the group of subjects with benign eye tumors had a lower mean of 1.08 mg/dL. dL (0.34-1.68 mg/dL). This result was statistically significant (p=0.042). According to a previous study by Dikovskaya et al., individuals with malignant and benign eye tumors had significantly greater quantities of Cystatin C in their tear fluid compared to the control group, yet there was no significant difference (p>0.05) (Dikovskaya et al., 2021).

The results of studies on cancer and benign tumors of other organs found that increased Cystatin C was associated with the development of malignancy. For example, the findings in a study related to the tear fluid Cystatin C in the serous tumor subgroup (p = 0.001). Cystatin C levels were higher in patients with epithelial ovarian cancer compared to benign ovarian tumors (Kolwijck, 2010). Although these observations support the hypothesis of Cystatin C involvement in cancer, its specific role in malignant disease remains inconclusive. Research specifically aimed to uncover the role of Cystatin C in tumor growth showed that this molecule exerts a complex set of effects that may result in the inhibition or promotion of tumor cell growth and spread (Leto, Crescimanno, & Flandina, 2018).

Conclusion

There was a significant difference in the tear fluid Cystatin C level between the individuals with and without eye tumors (p<0.001), with the mean tear fluid Cystatin C in the eye tumor group of 1.43 mg/dL. In addition, tear fluid Cystatin C levels differed significantly between malignant and benign eye tumor groups.

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