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Assessment of clinical signs associated with adenoviral epidemic keratoconjunctivitis cases in southern Japan between 2011 and 2014☆,☆☆,★

Koki Aoki [a,b], Gabriel Gonzalez [c], Rikutaro Hinokuma [d], Nobuyo Yawata [e,f,g], Masayuki Tsutsumi [a,b], Shigeaki Ohno [a,b], Nobuyoshi Kitaichi [a,b,*]

[a] Department of Ophthalmology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan
[b] Department of Ophthalmology, Health Sciences University of Hokkaido, Sapporo, Japan
[c] Division of Bioinformatics, Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan
[d] Hinokuma Eye Clinic, Kumamoto, Japan
[e] Department of Ophthalmology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[f] Singapore Eye Research Institute, Singapore
[g] Duke-NUS Medical School, Singapore

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ABSTRACT

Adenoviral epidemic keratoconjunctivitis (EKC) is a major cause of ocular morbidity worldwide and specific antiviral therapies are not available. EKC is primarily caused by *Human adenovirus D* (HAdV-D) types 8, 37, 53, 54, 56 and 64. Considering the genomic variation in HAdV-D, we hypothesized that clinical signs could be differentiated by virus type. The hypothesis was retrospectively tested with clinical signs recorded from 250 patients with ocular infections visiting an ophthalmological clinic in southern Japan between 2011 and 2014. The results showed that conjunctival opacity, corneal epithelial disorders and pre-auricular lymphadenopathy, were more frequently associated with EKC than other ocular infections. Furthermore, HAdV types 8, 37 and 54, caused corneal complications and longer infections significantly more frequently than infections by types 53 and 56 ($P < 0.05$). Our descriptive results supported that symptoms severity vary with the infecting type, however, further research is needed to improve diagnosis of EKC.

1. Introduction

Viruses in the *Human adenovirus* family (HAdV) cause sporadic infectious disease outbreaks worldwide and potentially lethal opportunistic infections in immunocompromised patients (Echavarria 2008; Gonzalez et al. 2019). Among these infections, epidemic keratoconjunctivitis (EKC) has a broad spectrum of clinical presentation and represents an ongoing threat to public health (Darougar et al. 1983; Gonzalez et al. 2019; Jawetz 1959; Jawetz et al. 1955). EKC is a major cause of ocular morbidity in developed and developing countries and efficacious therapeutic options remain to be developed (Aoki et al. 1979; Gonzalez et al. 2019). EKC is caused mainly by HAdV-D types 8, 19a (renamed 64), 37, 53, 54, and 56 (Aoki et al. 2008; Hage et al. 2017; Ishiko et al. 2008; Kaneko et al. 2011a; Kaneko et al. 2011b; Zhou et al. 2012); and HAdV-E type 4. For over half a century, types 8, 37 and 64, were considered the major causes of EKC outbreaks worldwide (Aoki et al. 2011; Aoki and Tagawa 2002; Guyer et al. 1975; Sprague et al. 1973); however, the recently recognized recombinant types 53, 54, and 56, have been characterized as the cause of EKC in Japan and other countries with a wide range of variation in the clinical signs exhibited during the infections (Gonzalez et al. 2019; Kaneko et al. 2009; Kaneko et al. 2011b). Although other adenoviral types have been isolated and associated with severe ocular infections, their characterization as EKC causative agents requires the assessment of the clinical signs and determination of the cornea involvement in such infections (Fujimoto et al. 2014; Gonzalez et al. 2019; Hashimoto et al. 2018).

EKC is defined as an ocular infection characterized by clinical signs including severe hyperemia, diffuse cellular infiltration, lacrimation, follicular conjunctivitis, pseudo-membrane formation and the formation

Abbreviations: EKC, epidemic keratoconjunctivitis; HAdV, human adenovirus; MSI, multiple subepithelial corneal infiltrates; IC, immunochromatography; CPE, cytopathic effects; PCF, pharyngoconjunctival fever; SPK, superficial punctate keratitis; PEE, punctate epithelial erosions; FK, filamentary keratitis.

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* Corresponding author. Tel.: +81-11-778-7575; fax: +81-11-770-5034.
E-mail address: nobukita@hoku-iryo-u.ac.jp (N. Kitaichi).

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of multiple sub-epithelial corneal infiltrates (MSI), and regional lymphadenopathy, such as mild swelling and tenderness of the pre-auricular nodes (Aoki et al. 2011; Darougar et al. 1983; Kaneko et al. 2008). Invasive infections of the cornea with a filamentous keratitis, and corneal erosion and ulceration can occur and persist for extended periods of time (Darougar et al. 1983; Meyer-Rusenberg et al. 2011). The burden of clinical disease arising from adenovirus outbreaks brings socioeconomic consequences to medical centers and communities (Hage et al. 2017; Sprague et al. 1973). In addition, adenoviral types in species HAdV-B, -C and -E have been characterized to establish latent infections in the eye, respiratory tract and intestinal tissue that could trigger nosocomial infections or recurrent symptoms over the years such as papillary conjunctivitis (Kaye et al. 2005; Lion 2014).

The genetic variation between HAdV types is defined by a variety of nucleotide differences at genomic level (Seto et al. 2011). Traditionally, serology was employed to distinguish types with the use of neutralizing anti-sera against each type, that reflected the genomic differences in epitope determinant regions in the surface hexon, penton base and fiber proteins (Crawford-Miksza and Schnurr 1996; Gahery-Segard et al. 1998). Nevertheless, the effects of other frequently recombined genomic regions remain less well characterized with respect to the impact on the disease course (Gonzalez et al. 2014; Gonzalez et al. 2015). It is noteworthy that recombination events have affected the serology of multiple adenovirus types with effects in their seroepidemiology, such as types 64 and 81 that are neutralized by anti-sera against type 19 and 48, respectively (Fujimoto et al. 2014; Zhou et al. 2012); thence, there is a growing inclusion of molecular methods in epidemiological studies to provide a broader interpretation of adenovirus species and types behind infectious outbreaks and their relation to demographic factors (Aoki et al. 2008; Aoki and Tagawa 2002; Barnadas et al. 2018; Kaneko et al. 2009; Kaneko et al. 2011a; Tsukahara-Kawamura et al. 2018).

As EKC is caused by more than a single differentiable adenoviral genome type, we hypothesized that genomic differences among types could have effects on the range of the intensity of clinical signs, symptoms and the course of the infections. Furthermore, we hypothesized these differences could also be reflected in the epidemiology of viruses causing ocular diseases; thence, we have retrospectively analyzed the clinical data collected in a clinic in the Kumamoto prefecture of southern Japan between 2011 and 2014 comprising 250 ocular infections with 136 confirmed adenoviral infections and 114 cases unrelated to adenovirus. The clinical records show variation in the exhibited clinical signs with severity varying according to the infecting adenoviral type.

2. Materials and methods

2.1. Patients treatment and data collection

The clinical signs and symptoms observed on first clinical presentation in patients attending an ophthalmology clinic in southern Japan during the period 2011–2014 were recorded for a total of 250 ocular infections. The age of patients ranged between 1 and 83 years old with an average of 39 years. After signs and symptoms were putatively attributed to EKC, immunochromatography (IC) kits, Adenocheck® (Santen, Japan) and Capilia Adeno Eye Neo® (Wakamoyo, Japan), were employed to determine the presence of adenovirus or not in the infection. In addition, the conjunctival scrapings were analyzed by PCR and partial sequencing targeting the hexon gene to identify the adenoviral type employing standard procedures, as described elsewhere (Hashimoto et al. 2018; Yoshitomi et al. 2017). Patients with confirmed adenoviral infections received corticosteroid treatments as eye-drops containing fluorometholone and chloramphenicol at 0.1% and 0.5% concentration, respectively, from the first visit to the conclusion of the infection.

This study was performed following the guidelines of the Ministry of Health, Labour and Welfare of the Japanese Government and the National Institute of Infectious Diseases (NIID), Tokyo which has performed the surveillance programs for infectious agents in Japan since 1980.

This study was approved by Institutional Review Board (No. 2016–002).

2.2. Cytopathic effects of HAdV-positive samples in A549 cells

Clinical samples were inoculated in A549 cells maintained in Eagle's minimal essential medium supplemented with 2% fetal bovine serum. The cultures were observed for 3 weeks for cytopathic effects (CPEs) of HAdVs.

2.3. Statistical analysis

R v3.6.0 (R Development Core Team R, 2011) was used to assess the statistical significance of frequencies and the association between different clinical signs. Fisher's exact test was used to assess the significance of differences in frequencies among infectious agents and observed symptoms. T test was used to assess the significance of the difference in duration of infections by type. χ^2 Test was used to assess the significance of the number of observed cases by age group in comparison to the expected distribution of cases according to the population distribution by age group for the Kumamoto prefecture (Official Statistics of Japan for 2011, https://www.e-stat.go.jp/en).

3. Results

3.1. Detection and identification of epidemic keratoconjunctivitis

Adenoviral infections were detected in 136 out of the 250 cases. Adenovirus was detected by IC in 112 cases at first visit (82%) and partial sequencing of hexon coding region for other 24 cases that were negative in the IC tests; however, 11 out of these 24 cases, were identified as adenovirus infections by IC during the follow up. The characterized cases were associated with HAdV-B 3 ($n = 8$, 5.9%), 11 ($n = 1$, 0.7%), 34 ($n = 2$, 1.5%), HAdV-E 4 ($n = 15$, 11%), HAdV-D 8 ($n = 19$, 14%), 37 ($n = 36$, 26.5%), 53 ($n = 9$, 6.6%), 54 ($n = 16$, 11.8%), 56 ($n = 28$, 20.6%) and 64 ($n = 2$, 1.5%) (Table 1). Strikingly, two types were detected less frequently by IC on the first visit: 8 (13/19, <68%) and 56 (20/28, <71%). These frequencies were lower when compared to the expected (>90%) sensitivity of the IC kits (Fujimoto et al. 2004), which indicated properties inherent to these viral types, such as the loss of an epitope allowing them to escape detection by the IC kits and/or lower number of viral copies in samples at the time of the initial testing.

The clinical signs and symptoms recorded during this study were palpebral conjunctival injection, bulbar conjunctival injection, conjunctival palpebral hemorrhage, lid swelling, chemosis, conjunctival opacity, corneal epithelial disorder, preauricular lymphadenopathy, ocular discharge, lacrimation, ocular pain, foreign body sensation and itching. During the considered period, in 114 (45.6%) of the 250 cases of ocular infection, adenovirus pathogens were not detected by IC or molecular methods and assumed to be caused by other infectious agents; such cases were recorded and used in this study as negative control to compare the frequency of adenoviral-associated clinical signs (Table 2). The frequencies of chemosis, conjunctival opacity, corneal epithelial disorders and preauricular lymphadenopathy, were significantly more frequent in adenoviral ocular infections than in non-adenoviral ocular infections ($P < 0.01$, Fisher's exact test). Other symptoms slightly more prevalent in adenoviral EKC, but lacking statistical significance in the considered sample size, were palpebral conjunctival hemorrhage ($P < 0.16$, Fisher's exact test), lid swelling ($P < 0.21$, Fisher's exact test), lacrimation ($P < 0.15$, Fisher's exact test) and foreign body sensation ($P < 0.38$, Fisher's exact test).

Table 1
EKC cases recorded in southern Japan between 2011 and 2014.

Species	Types	2011	2012	2013	2014	Total	%	IC (%)	Age distribution (%)		
									<15	15–64	64<
HAdV-B	3	2	-	5	1	8	5.9	7 (88)	12	88	-
HAdV-E	4	3	9	3	-	15	11.0	13 (87)	7	93	-
HAdV-D	8	-	4	7	8	19	14.0	13 (68)	-	77	23
HAdV-B	11	1	-	-	-	1	0.7	1 (100)	-	100	-
HAdV-B	34	0	2	-	-	2	1.5	2 (100)	-	100	-
HAdV-D	37	2	1	3	30	36	26.5	31 (86)	3	80	17
HAdV-D	53	-	6	1	2	9	6.6	8 (89)	-	100	-
HAdV-D	54	5	9	1	1	16	11.8	16 (100)	-	100	-
HAdV-D	56	5	12	7	4	28	20.6	20 (71)	-	96	4
HAdV-D	64	1	-	1	-	2	1.5	1 (50)	-	50	50
	Total	19	43	28	46	136		112 (82)	2	89	9

3.2. Clinical manifestations in adenoviral EKC

The clinical signs collected from 136 patients with adenoviral infections were assessed further by analysis for common patterns and differences among the associated adenoviral types. Recorded signs and symptoms are generally related with EKC and other adenoviral ocular infections, such as pharyngoconjunctival fever (PCF), which usually shows respiratory symptoms which likely limits the number of patients seeking treatment in an ophthalmology clinic. Due to such overlap of clinical signs between these pathologies, some cases considered as possible EKC at the first visit were revised to a different diagnosis during the development and resolution of the infections. Despite cases by types 3, 4, 11 and 34 exhibited palpebral and bulbar conjunctival injections, the lack of chemosis and corneal epithelial complications led us to consider these infections as conjunctivitis; moreover, these types are more frequently related to acute hemorrhagic conjunctivitis (AHC) and pharyngoconjunctival fever (PCF) (Aoki et al. 1982; Uchio et al. 1999). Furthermore, the duration of the infections from onset to resolution by non-HAdV-D types (10.9 ± 2.96 days) was significantly shorter than infections by HAdV-D types (16.60 ± 7.82 days) ($P < 2 \times 10^{-4}$, t test). In addition, the average time required by adenoviral isolates to exhibit cytopathic effect (CPE) varied among types (Fig. 1). Notably, the CPE from types 8 and 54 were positive in only 2 out of 19 (11%) and 6 out of 16 (38%) cases, respectively; whereas other types were positive in 93 out of the remaining 101 cases (92%) (Table 3).

Table 2
Comparison of ocular infections by adenovirus and other infectious agents.

	Adenoviral Infection	Negative for HAdV	Fisher's exact test	
			Odds ratio	*P*
Cases	136	114	1.00	1.00
Palpebral conjunctival injection	136	114	1.00	1.00
Bulbar conjunctival injection	134	114	1.02	1.00
Palpebral conjunctival hemorrhage	104	65	0.75	0.16
Lid swelling	73	45	0.74	0.21
*Chemosis	8	-	0.00	0.01
*Conjunctival opacity	108	43	0.48	7.2×10^{-4}
*Corneal epithelial disorder	30	2	0.08	5.4×10^{-6}
*Preauricular lymphadenopathy	46	12	0.31	5.7×10^{-4}
Discharge	129	111	1.03	0.93
Lacrimation	97	60	0.74	0.15
Ocular pain	58	48	0.99	1.00
Foreign body sensation	76	52	0.82	0.38
Itching	46	41	1.06	0.80

* Symptoms shown in adenovirus infections with significantly higher frequency than in cases negative for adenovirus.

All adenovirus infections evidenced palpebral and bulbar conjunctival injections (Table 3); however, only 22.1% of the infections were characterized with corneal epithelial complications, such as multiple subepithelial infiltrates (MSI), corneal erosion (PEE), superficial punctate keratitis (SPK), punctate or filamentary keratitis (FK). The types associated with corneal disorders were 8, 37, 53, 54 and 56. Strikingly, these types were the most common cause of severe ocular infections during this study (108/136 infections, 79.4%); therefore, the analysis of the clinical signs characterized for EKC in the present study focused on these types.

A strong association of types 8, 37, 53, 54, and 56 with ocular infections with severe symptoms was evident and the reason that prompted patients to visit the clinic. These types were noteworthy in their manifestation of corneal complications in 30 out of 108 cases (27.8%) (Table 3). Nevertheless, types 8, 37 and 54, were associated with a two-fold significantly higher frequency of corneal complications (33.80% of combined cases) in comparison to types 53 and 56 (16.22% of combined cases) ($P < 0.05$, Fisher's exact test) (Fig. 2). However, the relatively small number of cases related to type 53 limits the interpretation of its severity. Also, the presence of palpebral conjunctival hemorrhage in cases with corneal involvement was statistically more frequent in types 8, 37 and 54 than in cases attributed to types 53 and 56 ($P < 0.05$, Fisher's exact test) (Fig. 2).

4. Discussion

Adenoviral infections are a frequent cause of medical concern due to opportunistic infections and the possibility of nosocomial outbreaks (Tsukahara-Kawamura et al. 2018). Although the isolation of adenovirus from an ocular infection is frequently associated with EKC, not all cases correspond to the etiology of the disease; for instance, some of the early stages of severe acute hemorrhagic conjunctivitis and PCF can be misdiagnosed as EKC. Our primary objective was to define more accurately the EKC presentation by summarizing and assessing the frequency of associated clinical signs and symptoms. The observed cases pointed to the presence of conjunctival opacity, corneal epithelial disorders and preauricular lymphadenopathy, as signs significantly more frequently associated with EKC than with other ocular infections, such as bacterial or other viral agents involved in ocular infections, consistent with previous work (Kaneko et al. 2008). The conjunctival opacity and corneal epithelial disorders in EKC cases, such as nummular opacities and corneal infiltrates, are characteristics that set this infection apart from others and the debilitating sequelae can last from months to years (Hillenkamp et al. 2001). Histopathological evidence of focal biopsy revealed corneal subepithelial infiltrates of lymphocytes, histiocytes and fibroblasts, in combination with disruption of collagen fiber in the Bowman layer (Lund and Stefani 1978). We hypothesize that the frequency of severe clinical signs by type was related to the number of days that the infections lasted or required to show CPE; although the latter could be explained by properties of the used cell lines, the

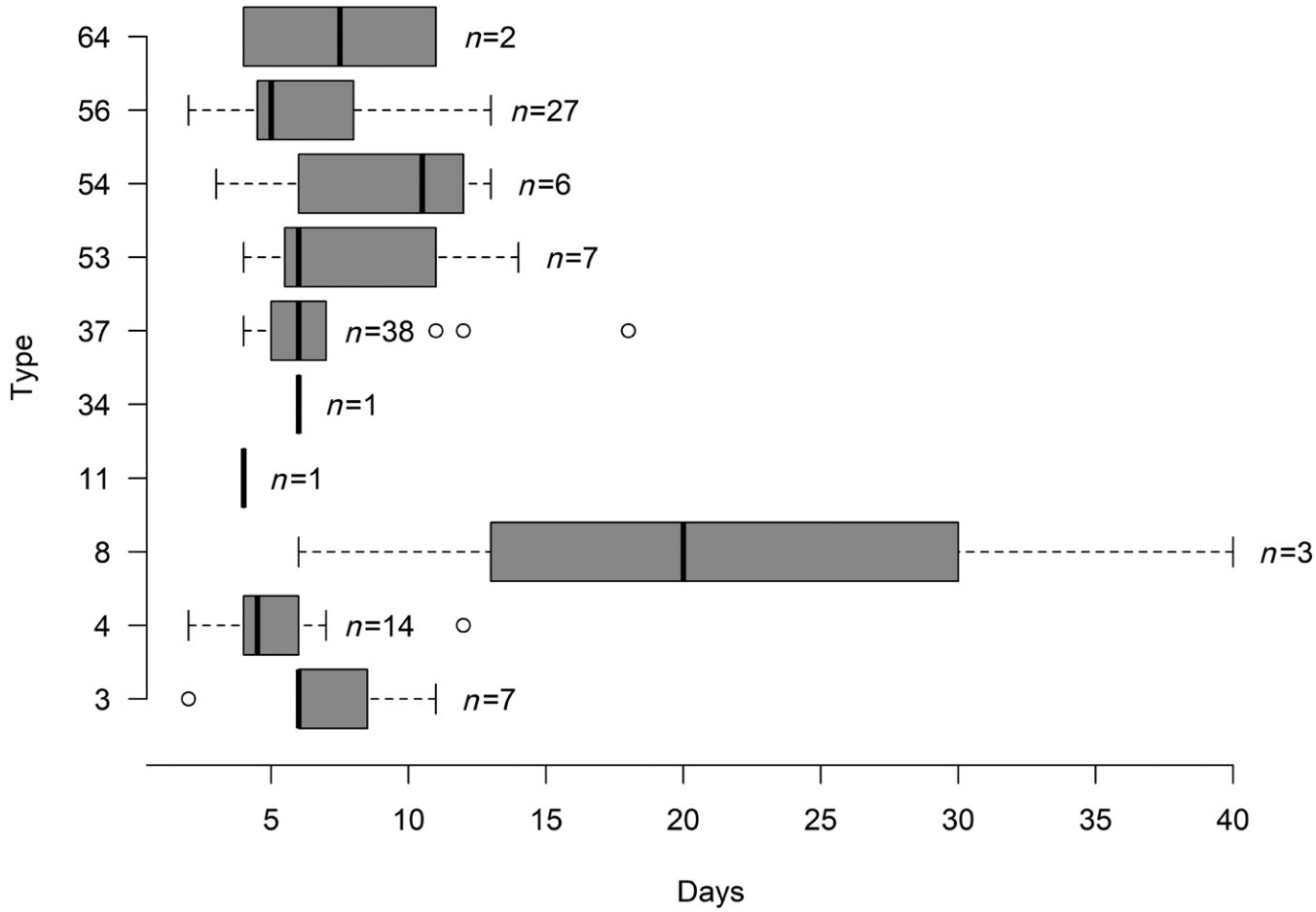

Fig. 1. Days to cytopathic effects per HAdV type. The boxplot shows per HAdV type the distribution of the number of days to CPE. Vertical axis shows the type and horizontal axis shows the number of days. The vertical line inside the box shows the average of days among all samples per type. Next to each box the number of samples is indicated.

persistent viral replication presumed to occur during infections with longer duration could potentially explain the immune-mediated lymphadenopathy. Interestingly, experimental results showed that adenoviral proteins secreted by infected cells block receptors in non-infected lymphoid cell lines and leukocytes, thus suppressing activation and cytotoxicity of natural killer cells (Martinez-Martin et al. 2016;

Table 3
Symptoms summarized based on adenoviral types.

	Identified Adenoviral Type											Distribution symptoms by age (%)		
	3	4	8	11	34	37	53	54	56	64	Total	0–14 y. o.	15–64 y. o.	65 y. o. and over
Cases	8	15	19	1	2	36	9	16	28	2	136	2	89	9
Average duration (days)	7	12.7	16.6	10	-	15.4	19	18.2	16.8	9	15.6 ± 7.4	13.7 ± 6.4	16.2 ± 7.0	14.3 ± 9.7
Palpebral conjunctival injection	8	15	19	1	2	36	9	16	28	2	136	11	81	8
Bulbar conjunctival injection	8	15	18	1	2	36	9	16	27	2	134	11	81	8
Palpebral conjunctival hemorrhage	5	13	18	1	2	26	5	11	21	2	104	11	82	7
Lid swelling	2	6	12	1	2	26	3	8	13	-	73	11	77	12
Chemosis	-	-	2	-	-	3	-	1	2	-	8	12	50	38
Conjunctival opacity	5	14	16	-	1	27	9	12	22	2	108	13	81	6
Corneal epithelial complications[+]	-	-	6	-	-	12	1	6	5	-	30	17	70	13
% SPK[*]	-	-	*66*	-	-	*16*	*100*	*17*	*20*	-	*30*			
% PEE[*]	-	-	*34*	-	-	*33*	-	-	-	-	*20*			
% FK[*]	-	-	-	-	-	*8*	-	-	*20*	-	*7*			
% MSI[*]	-	-	-	-	-	*43*	-	*83*	*60*	-	*43*			
Preauricular lymphadenopathy	2	6	7	1	-	13	2	4	11	-	46	11	87	2
Discharge	8	15	19	1	2	31	9	15	27	2	129	10	81	9
Lacrimation	4	14	14	-	2	25	8	10	19	1	97	9	82	9
Ocular pain	4	5	9	-	-	17	4	5	14	-	58	10	81	9
Foreign body sensation	2	7	8	1	2	24	5	7	19	1	76	8	85	7
Itching	1	6	5	-	-	12	5	6	10	1	46	7	89	4
Positive CPE (%)	88	93	11	100	50	97	78	38	93	100	74	3	89	8

[+] Despite some cases presented multiple corneal epithelial complications, in each case the reported complication was the most severe.
[*] SPK: superficial punctate keratitis, PEE: punctate epithelial erosions, FK: filamentary keratitis, MSI: multiple subepithelial infiltrates.

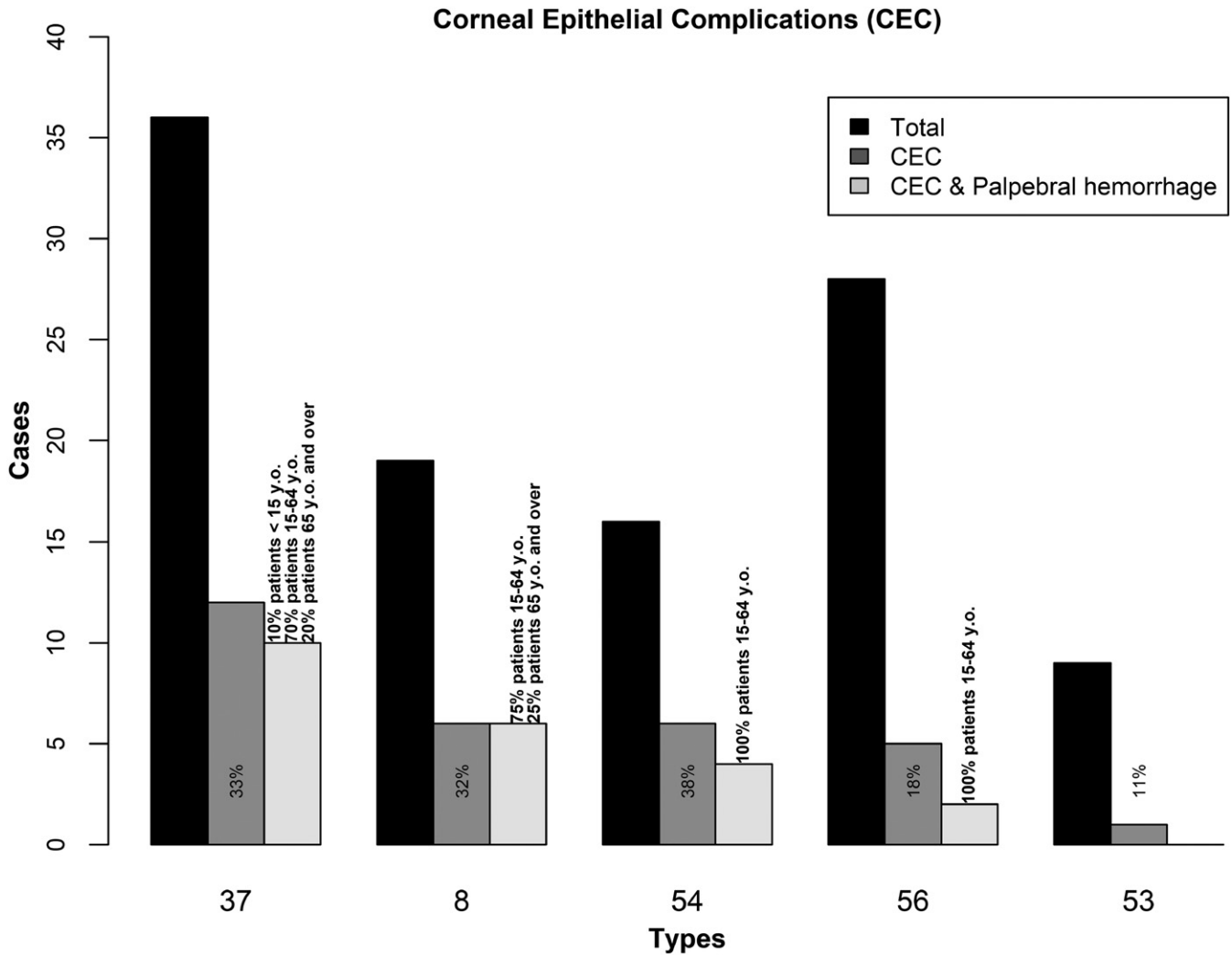

Fig. 2. Number of cases by type with corneal epithelial complications (CEC). The horizontal and vertical axes show the types and number of cases per type, respectively. Bars represent per type the total of cases (black), cases with CEC (gray) and cases presenting CEC and palpebral hemorrhage (light gray). The percentage of cases by type with CEC is shown in the gray bar. The percentage of cases by age group showing CEC and palpebral hemorrhage are shown above the light gray bars.

Windheim et al. 2013); we hypothesize that such regulatory activity help perpetuate the infection so as to evade the immune responses for longer periods in HAdV-D than in other adenoviral species.

During this study, the most frequently identified adenoviral types in ocular infections corresponded to 8, 37, 53, 54 and 56. Those patients with confirmed adenoviral infections on the first visit received corticosteroid therapy to reduce the risk of corneal involvement. Such a policy follows previous reports demonstrating that topical corticosteroid treatment reduces the incidence of corneal complications (Gonzalez et al. 2019; Laibson et al. 1970; Sugiura 1959). In this study, only 27.3% of cases by HAdV-D types (30/110) developed corneal involvement, while other previous studies focusing on similar adenoviral types provided evidence of corneal involvement in up to 72% of cases (16/22), suggesting corticosteroids are useful to prevent development of corneal complications (Sugiura 1959). However, the usage of topical corticosteroid should be restricted to complicated cases, as animal studies have shown its use can increase the replication rate of adenovirus in the conjunctiva and extend the duration of the infection (Romanowski et al. 1996; Romanowski et al. 2002). Therefore, the correct and prompt identification of the infectious agent in the ocular infection is important to address properly an effective treatment.

The false-negative detection rate of IC observed for types 8 and 56 raises the possibility of properties inherent to these types that affect the detection potential of the point-of-care diagnostic serology kits. The lower limit of detection by the IC kits is 10^4 viral particles per ml of specimen (Fujimoto et al. 2004), therefore, a high false-negative detection rate could imply low viral loads in the conjunctival tissue infected with types 8 and 56 when the samples were taken. An alternative hypothesis to explain the false-negative rate is attributing the observed lower detection rates to a lower affinity to the monoclonal antibody originally developed targeting *Human adenovirus C*, which is used in the IC kit (Fujimoto et al. 2004). In either case, considering that this study found the most frequent cause of severe ocular infections were types in the HAdV-D, improvements to detection tools are suggested to be achievable by targeting types in this species.

This study identified cases caused by the same adenoviral type that presented differences in the development of symptoms and even in the frequency of developed corneal epithelial disorders. These variations in clinical presentation point to conditions external to the virus such as the host immune response. We hypothesize the genomic differences between types affected the host immune reaction reflected in the variations observed in the severity of the symptoms associated with types 8, 37 and 54, against those associated with 53 and 56. Furthermore, 56 has been characterized as the product of recombination events among types 15 and 9 (Kaneko et al. 2011b); while 53 was characterized as the product of recombination events among types 8 and 37 (Kaneko et al. 2011a), which are frequent source of severe ocular infections (Aoki and Tagawa 2002). Therefore, other factors than the identity

of the recombinant parents are suggested to be involved in the pathogenic characteristics of novel recombinant types; moreover, the recombined genomic loci of novel viral types are relevant to disease severity, length, and detectability of infections (Gonzalez et al. 2019).

Despite differences among patients were out of the scope due to the retrospective nature of this study, we speculate whether demographic characteristics of Kumamoto prefecture or clinical histories of the patients could be related to the severity and resolution of the infections. Furthermore, in regard to the distribution of the 1.8 M population in Kumamoto prefecture with 13.7%, 60.5% and 25.8% in the age groups under 15 y. o., between 15 and 64 y. o. and over 65 y. o., respectively, the distribution of cases was significantly higher in the middle age group than the expectation (Table 1) ($P < 2.5 \times 10^{-11}$, χ^2 test). Such an observation is consistent with reports of adenoviral infections in infants more frequently associated to respiratory diseases (Rocholl et al. 2004), also, differences in eye anatomy and immune response between young and adult patients lead to variations in the epidemiology (Augusteyn 2010; Simon et al. 2015). Consistently, with exception of chemosis, recorded symptoms were manifested in middle age patients with higher frequency (>70%) than in the other age groups (Table 3), in particular patients with CEC and palpebral hemorrhage (Fig. 2). The cases considered in the current study were assumed to be primary infections and the presence of previously acquired antibodies or latent infections in immune competent patients was not assessed; however, the presented evidence opens the question of whether the observed variations in severity of symptoms could relate to previous infections that primed the patients in certain degree against types related to ocular infections as it has been shown for other types (Barouch et al. 2011).

5. Conclusion

This study has summarized and assessed the clinical signs associated with adenoviral ocular infections. The results demonstrate that the variety of types associated with EKC varies in the intensity of signs, symptoms and the length of the infections. This study faced limitations in the scope, therefore, the application of these observations into improvements in diagnosis and treatment of EKC require further study of the properties behind these variations. Nevertheless, consideration must be taken in outbreaks and nosocomial infections of HAdV-D types to provide prompt topical treatment of ocular infections and avoid damage to the ocular tissue or lasting visual impairment. Also, the false-negative rate observed in detection and identification methods suggests the need for improvement to such IC assays that consider the variety and the epitope stability of the infectious agents.

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