Studies on Epidemic Cases of Pharyngo-conjunctival Fever due to Adenovirus Type 3 in Nagasaki

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Abstract

In summer, 1966, the patients of acute respiratory illness characterized by elevated fever, pharyngitis and / or conjunctivitis were observed among school-children at Nagasaki city. All patients had history of swimming to pool on end of July, and the number of cases increased rapidly on beginning of August.

The etiologic diagnosis of the epidemic was made by virus isolation and serologic investigation on patients visited hospitals, and adenovirus was isolated in HeLa cell cultures from those patients and virus isolation rate was 41% of 54 cases. Isolated viruses were all identified as type 3 using a rabbit antiserum against adenovirus type 3.

A significant rising of antibodies on complement-fixing and neutralizing tests was each 77 % and 94 % of acute and convalescent sera collected from 54 cases. Thirty-four of 54 cases was diagnosed by virus isolation or serologic investigation as adenovirus type 3 infection.

Introduction

Pharyngoconjunctival fever being a common communicable disease of school-children characterized by pharyngitis, conjunctivitis and fever is caused by several types of adenovirus, in which it has been known type 3 to be most causal. There are also the paper recording isolation of type 1,2,5,6,7, and 14.

And now, a acute respiratory illness which was considered pharyngo-conjunctival fever out-breaked among school-children at Nagasaki city in summer, 1966.

The present paper describes the definition of causal agent of pharyngo-conjunctival fever children in the above description, namely the isolation and identification of adenovirus and serologic evidence. Such investigation is the first one in this laboratory or Nagasaki city.

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Materials and Methods

Virus Isolation: The throat and anal swabs of those out-patients who visited hospitals to chiefly complain fever in end of June to beginning of August, 1966, were collected in tube containing 2 ml of broth and a mixture PC 1000 unit/ml with SM 1000 γ/ml for use of virus isolation. HeLa cells were cultured in a stationary rack at 37°C for 2-3 days in tubes consisting of Hank's balanced salt solution containing of 10% bovine serum and 0.5% lactalbumin hydrolysate. Monosheets of cell cultures having been formed, the growth medium was changed for maintenance medium YLH (Hank's balansed salt solution containing of 0.1% yeast and 0.5% lactalbumin hydrolysate) cantaining of 2% bovine serum.

Collected specimens were diluted in Puck's balanced salt solution containing of a mixture PC 1000 unit/ml with SM 1000 γ/ml to 2-4 times. After they were stirred fully, then left at 4°C for 1-2 hours and subjected to centrifugation at 10,000 rpm for 30 minuits.

Then 0.2 ml amount of these supernate brought out was inoculated into each 2-4 tubes prepared, and which were observed microscopically daily or every other day for their cytopathogenic effect (CPE) for 2-3 weeks.

While, medium fluids were changed at intervals of 4-6 days. The test was considered to be negative after three blind passages.

Neutralization Test: Virus isolated from no. 4 patient was diluted in Puck's balanced salt solution (BSS) to produce CPE in HeLa cells on 3-4 days. Serial twofold dilutions of serum were prepared in Puck's BSS from a dilution of 4 to 256 times. Then 0.3 ml of virus dilution and 0.3 ml of serum dilution were mixed. The serum-virus mixture having been held at 37°C for 1 hour, 0.2 ml amount of the mixture were inoculated into each 2 tubes of HeLa cell. The inoculated cell were cultured at 37°C for 7 days and observed microscopically daily for their CPE. The maximal serum dilution which inhibited a appearances of CPE over 24-48 hours after CPE appearances of control virus, was taken as the neutralizing titer of the serum tested.

Complement Fixing Test: The antigen for complement fixing test was prepared from HaLa cell cultures infected with the prototype strain of adenovirus type 3. HeLa cells were cultured at 37°C in bottles using Hank's solution containing 0.5% lactalbumin hydrolysate and 10% bovine serum. After cell had grown in a monolayer, the culture medium was removed and the cell sheet was rinsed twice with Puck's solution. The cultures were inoculated with a great deal of the virus so as to produce complete destruction of the cells in 7 days of incubation. Then, cultures were frozen and thowed 5 times and subjected to centrifugation at 2,000 rpm for 20 minuits.

CF antigen unit was determined by box titration of the supernate after to be heated at 56°C for 30 minuits using anti-adenovirus type 3 serum immunized in rabbits.

Four units of antigen was employed for the tests. Serial twofold dilution of serum were prepared in VBS (Veronal Buffer's solution) from a dilution of 4 to 128 times after to be heated at 56°C for 30 minuits. And the tests were carried out as follow. To 0.02 ml of serum dilution, 0.02 ml of 4 units antigen prepared in the above method was added and to them 0.04 ml of complement was added.

The mixture were incubated at 4°C for 24

hours and then 0.04 ml of the hemolytic system consisting of 0.02 ml of a 2.5% sheep red blood-corpuscle suspension and 0.02 ml of a 20 units hemolysin was added to each holls. The mixture having been incubated at 37°C for 30 minuits and then at 4°C for 60 minuits, the test was read the titer to be shown the maximal dilution of serum producting about 50 % hemolysis.

Results

Clinical Observation: All patients had history of swimming to Aba-pool on the 20th of July to 11th of August, and to Higashi high school's pool on 23-25th July. And then, the number of cases increased rapidly starting July 25 and reached a peak on 1 of August. (See Fig. 1) The total number of case in this epidemic was not investigated and also whether the disease spread or not to the families of children, to other families, and also to adjoing districts. After August 10 a occurrence of patients decreased remarkably and since then it continued sporadically untill on end of August.

As seen Fig. 2 and Fig. 3, we encountered 54 cases of out-patients diagnosed clinically as viral respiratory disease. Age of patients was 1 to 14 years old without distinction of sex, and a great number of them were school-children and had a temperature of 38-39°C which had continued for 3-5 days. Pain in the throat and head ache was complained in about 50% of the cases, and diarrhea and abdominal pain in about 30%

Pharyngitis was almost observed in all cases and conjunctivitis in about 50% of the cases. Although it was a few cases, there were children showing meningial sign. Never one case was observed with exanthem.

Virus Isolation and Identification: As seen in Table 1 and Fig.4, 30 strains of adenovirus were isolated from 84 specimens collected from 54 patients, and from 20 cases aged 6-14. Because of a few specimens for virus isolation we can not say unfortunately that virus was more readily isolated from fecal than throat specimens, or from which were taken in 3 days after the onset of illness. The clinical observations of the disease and the CPE of the isolates in HeLa cell cultures presumed that isolated agents might be adenovirus. And then, isolated viruses were

Fig. 1 The appearance of cases of paryngoconjunctival fever in Nagasaki.

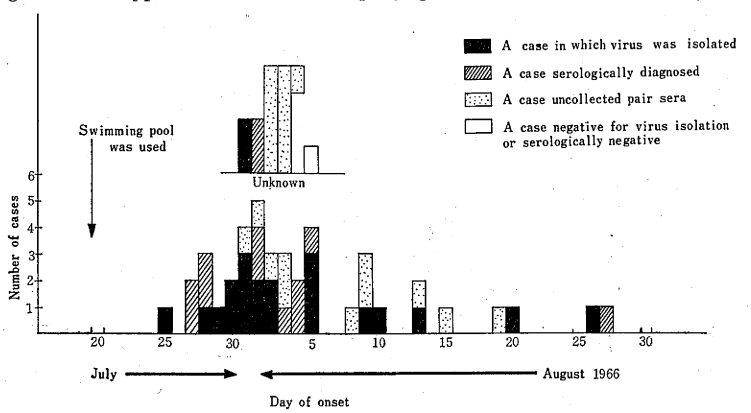


Fig. 2 Clinical Observation

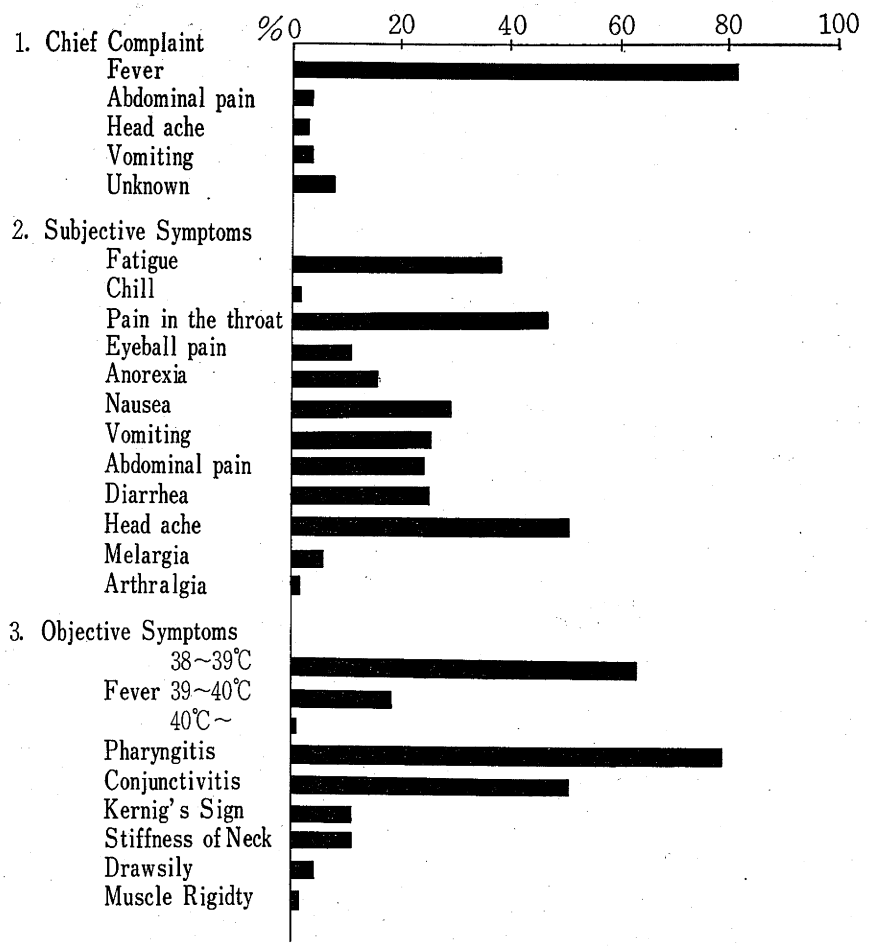
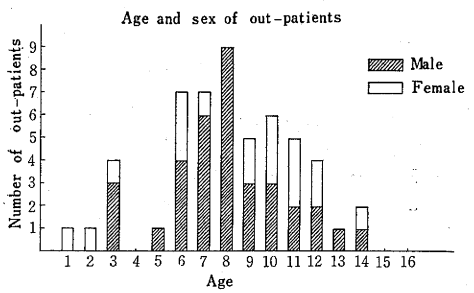


Fig. 3



all identified by neutralization test as adenovirus type3 using a rabbit antiserum against adenovirus type 3.

Serological Investigation: Complement-fixing and neutralizing tests were carried out for 17 pair sera and 22 single sera of 54 cases, and four-fold or greater rise in antibody titer was taken as significant. As seen in Table 2, the positive cases of CF and Neut. tests were

Fig. 4

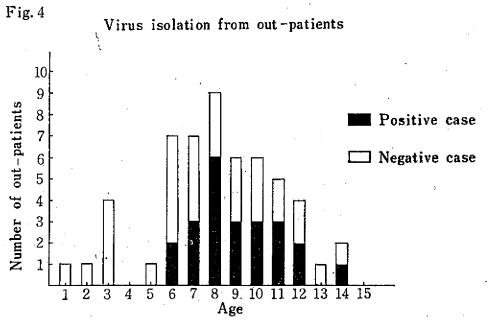


Table 1 Isolation of adenovirus type 3 from patients

Specimen	Days after onset							Total
	1	2	3	4	5	6	7	
Throat swab	1/3	2/12	8/17	3/8	2/5	0/4	0/3	16/52 (31%)
Feces		1/2	7/12	3/5	3/6	0/3	0/4	14/32 (44%)

Results are expressed as number of positive samples per examined.

Table 2 Summarized results of virus isolation and serological tests with acute and convalescent sera.

Tests	Patients with positive cases	
	Number	%
CFT	13/17*	77
NT	16/17*	94
VI	22/54	41

VI=Virus isolation
CF=Complement fixation test
NT=Neutralization test
* Four-fold or greater rise in antibody titer is taken as significant.

Table 3 Virus isolation and adeno 3 antibodies rises with 2 serologic tests

Virus Isolation	Pattern of Response		Number of Positive	
	Neut.	CF	NO.	%
P	P	P	8	47
P	P	N	1	6
P	N	P	0	0
P	N	N	0	0
N	P	P	4	24
N	P	N	2	11
N	N	p	1	6
N	N	N	1	6
Totals			17	100

P=Positive, that is, for-fold or greater rise in antibody titer is taken as significant.
N=Negative

each 13 of 17 pair sera (77%) and 16 of 17 (94%). Fig. 5 and Fig. 6 are showing the rising of antibodies associated with days after onset (See Discussion). Table 3 is showing

Fig. 5 and 6 Antibody titers of patients with pharyngo-conjunctival fever

Fig. 5

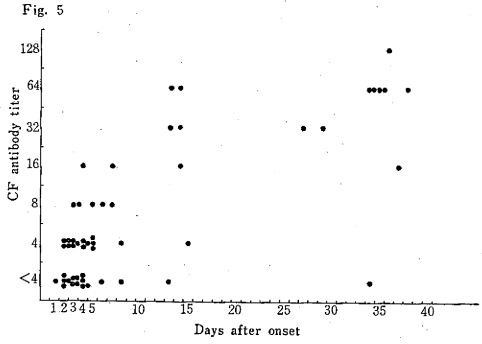
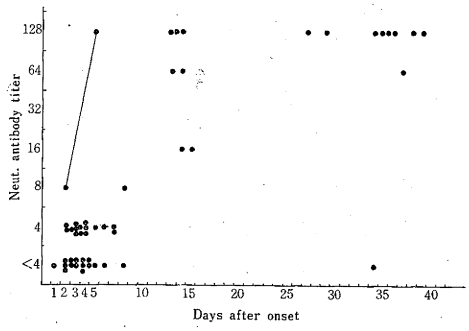


Fig. 6



the relationship of virus isolation and adenovirus type 3 antibody rising for serologic tests with regard to the cases of 17 pair sera collected.

The number of case to be tested positive with both virus isolation and antibody rising for two serologic tests, were 8 of 17 cases (47%). The remainder cases except for one case were positive on either virus isolation or serologic confirmation. That case to be tested negative virologically was the case suspected clinically pharyngoconjunctival fever because of having history of swimming to

Table 4 Serologic response and virus isolation of patients with pharyngo-conjunctival fever.

NO.	Age	Sex	Days after Onset	Antibody Titer		Virus Isolation
				Neut	CF	
1	11	♀	4		<4	Adeno 3
2	9	♀	3		<4	Adeno 3
4	11	♀	3 6	4	8	Adeno 3
5	9	♀	3 4	4	4	Adeno 3
8	8	♂	3 8	<4 4	<4 8	Adeno 3
9	7	♀	7 39	128	64	(−)
11	12	♂	3			Adeno 3
13	12	♀	2	4	4	Adeno 3
14	9	♀	2 36	4 128	<4 128	(−)
15	8	♂	2 34	<4 128	<4 64	Adeno 3
16	3	♂	2 34	<4 <4	<4 <4	
17	8	♂	3			Adeno 3
18	9	♂	2 5	8 128	4 4	(−)
20	11	♂	3 35	4 128	8 64	Adeno 3
21	8	♂	8 35	8 128	4 64	Adeno 3
22	9	♀	1			Adeno 3
26	7	♀	5 37	<4 64	4 16	Adeno 3
30	10	♂	7 38	4 128	16 64	(−)
31	11	♂	4	4	16	(−)
34	6	♀	6 29	<4 128	<4 32	(−)
37	8	♂	14	16	64	(−)
38	8	♂	5			Adeno 3
39	8	♂	Unknown Unknown	<4 16	4 4	(−)
40	7	♂	5			Adeno 3
44	10	♂	3			Adeno 3
45	8	♂	4 27	4 128	4 32	Adeno 3
46	11	♀	5	4	8	(−)
47	7	♂	2			Adeno 3
48	8	♂	Unknown	8	32	
49	10	♂	4 13	<4 128	<4 <4	Adeno 3
50	6	♂	4 13	<4 128	<4 32	Adeno 3
51	6	♂	3 13	4 64	4 64	Adeno 3
52	14	♂	3 14	4 64	<4 16	Adeno 3
54	7	♂	3 14	<4 128	4 32	

Aba-pool and the pharyngitis occuring in 5-6 days after going to swim. (See Discussion)

As seen in Table 4, 30 of 54 cases were diagnosed by virus isolation or serologic investigation as pharyngo-conjunctival fever caused by adenovirus type 3.

Discussion

Causal agent of this epidemic which outbreaked among school-children having gone to swimming pool, was made distinct by virus isolation and serological tests. Clinically, it was much the same as other reports that fever and pharyngitis were almost observed in all cases, and conjunctivitis in about 50% of the cases. In addition to their symptomes, gastrointstinal symptomes were observed in about 30 % of the cases. Hirayama's report (1962) have emphasized the severe gastrointestinal symptomes in pharyngoconjunctival fever epidemic. Although there was one case complaining severe gastro-intestinal symptomes with typical pharyngitis, virus isolation and serologic evidence were negative. But because of having gone to Aba-pool on July 24 as same other children to be positive virologically, (See Table 4), it was considered that the case was infected by adenovirus type 3.

Camplaining of the gastrointestinal symptomes without respiratory infection's sign was not only one case. While other one case complaining severe abdominal pain with mild fever was also negative virologically. Onset of this case was in August 27 (See Fig. 1).

Other one case with conjunctivitis and pharyngitis onsetting in August 26 had swum to Aba-pool and was positive virologically. But it was also considered that he may have been infected by contact with other infectious children because of indistinctness of his swimming day.

In typing isolated viruses, Neutralizing tests were employed and rabbit antisera against adenovirus typs 3 and 7, which were most causal type of pharyngoconjunctival fever diagnosed clinically, was used as well. In the result, isolated viruses were all identified as type 3. This finding was further confirmed by rising of antibodies against type 3.

As seen in Fig. 5 and 6, CF antibody began to rise in 5 days after onset, Neut. antibody in 8 days, and generally reached a maximum in about 2 weeks. One case showing that Neut. antibody rised 8 to 128 titer in 4 days after onset, was negative with both virus isolation and CF test, but appeared typical pharyngoconjunctival fever clinically.

It was considered that Neut. antibody titer 8 was due to old infection and such titer level was unable to defend against the virus attack in this epidemic. That is to say, the case was so readily infected with this epidemic as the Neut. antibody reached rapidly to 128 titer in early stage.

Other one case showing that both CF and Neut. antibodies did not rise in 34 days after onset, appeared typical pharyngoconjunctival fever clinically. Generally, CF antibody appeared somewhat earlier than Neut. antibody, but showed it's being difficult to rise remarkably.

A significant antibodies rising were 77% and 94% of 54 patients by CF and Neut. tests respectively, The results presented here also indicate the usefulness of the Neut. test on patients sera.

And now, virus isolation from pool-water were not accomplished, but Aba-pool and Higashi high school's pool were considered

to source of this epidemic, as the disease outbreaked among school-children having gone to these pools. And the number of children infected in this epidemic may have been much more than these cases tested.

Well, this description was reported on 44th Nagasaki Medical Associotcon and 18th Nishinihon Infectious Disease Association.

Summary

It was found that the epidemic of pharyn goconjunctival fever outbreaked at Nagasaki city in August, 1966 was due to adenovirus type 3. Clinically, there was not to pay a special attention to these patients. Adenovirus was isolated from 30 strains 54 cases, and isolated strains were all identified as type 3.

A significant antibody rising on these patients were 77% and 94% of 54 cases by CF and Neut. tests respectively.

The results presented here also indicates the usefulness of the Neut. test on patients sera. Thus, one case possesing Neut. antibody due to old infection have been infected also in this epidemic. Aba-pool and Higashi high school's pool were considered to source of this epidemic.

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長崎市内に発生したアデノウイルス3型に因る咽頭結膜熱の流行，松尾幸子，新城澄子，長崎大学風土病研究所ウイルス学部（主任：福見秀雄教授）

総　　括

1966年7月下旬から8月上旬にかけて 長崎市内の学童の間で咽頭結膜熱様疾患の多発が気付かれた．38°C以上40°Cに及ぶ発熱を主訴とし，咽頭炎，結膜炎を伴う患者が50%以上に認められ，下痢，嘔吐などの消化器症状をも伴っていた．市内の病院を訪れた54名の外来患者からウイルス分離検体として，咽頭拭液及び糞便を採取し，HeLa 細胞を用いた組織培養法により，患者54名中22名(41%)，検体別では咽頭拭液52件中16株(31%)，糞便32件中14株（44%）計30株のウイルスを分離することが出来た．臨床像及び HeLa 細胞による細胞変性効果からアデノウイルスが推定され，アデノウイルス3型の免疫血清を用いて，中和反応による同定試験を行なった結果，分離ウイルスはすべてアデノウイルス3型であることを確認した．又，血清学的検索を行なう為に，患者の急性期及び回復期のペア血清を採取できた17件について，補体結合試験及び中和試験を行なった結果，補体結合抗体では17件中13件(77%)，中和抗体については17件中16件（94%）にアデノウイルス3型に対する有意の抗体上昇を認めた．ウイルス分離陽性或は血清抗体陽性であった患者の殆んどは，長崎市内の南部にある網場プール及び東高校プールで7月20から8月11日の間に遊泳の形跡があり，この咽頭結膜熱の病因はプールを介するアデノウイルス3型に因る感染である事を証明した．