The incidence of anti-ATLA (antibodies to adult T-cell leukemia-associated antigen) among hemodialysis patients in the Kagoshima district with special reference to blood transfusion

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<abstract>

The incidence of anti-ATLA was investigated in 234 patients with chronic renal failure under hemodialysis. The results obtained were as follows. 1) The seropositivity of the patients (33.8%) was higher than that of healthy residents (11.7%) and blood donors (6.9%) in the Kagoshima district, an ATL-endemic area ($p < 0.01$). 2) There was no correlation between the duration of hemodialysis and anti-ATLA positivity. 3) The incidence of anti-ATLA increased with the rise in the number of blood transfusions given ($p < 0.05$). 4) The seropositivity of patients without episodes of blood transfusion was also higher than that of healthy residents in the Kagoshima district ($p < 0.05$). The data suggested that HTLV-1 was transmissible by blood transfusion, and that therefore it seems necessary to carry out mass-screening of blood units to prevent the spread of HTLV-1 infection in ATL-endemic areas, as soon as possible. Further future studies to clarify why the seropositivity of patients without blood transfusion was statistically high may also be necessary.

鹿児島県血液透析患者における抗 ATLA 抗体の検索 —特に輸血との関連について—

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輸血を受ける機会の多い血液透析中の慢性腎不全患者 234 例について、血清抗 ATLA 抗体を測定、HTLV-I 感染と輸血との関連について検討し、以下の結果を得た。1) 鹿児島県下の血液透析患者の抗 ATLA 抗体陽性率は 33.8%で、鹿児島県一般健常人の陽性率 11.7%より高率であった ($p < 0.01$)。2) 血液透析患者における抗 ATLA 抗体陽性率は、鹿児島県一般健常人にみられる加齢に伴う増加を示さなかった。3) 血液透析患者における抗 ATLA 抗体陽性率と透析期間との間には、有意の相関は認めなかった。4) 輸血の有無と抗 ATLA 抗体陽性率(輸血のある群の陽性率 37.7%、輸血のない群の陽性率 25.0%)との間には、統計学的有意差は認め難かったものの、輸血回数の増加に伴い、抗 ATLA 抗体陽性率は有意に ($p < 0.05$) 増加した。5) 輸血歴のない群での抗 ATLA 抗体陽性率も、鹿児島県一般健常人に比べ有意に ($p < 0.01$) 高く、輸血以外の因子の存在も示唆された。

今回の検討では、輸血歴のない患者群での抗 ATLA 抗体陽性率が、鹿児島県一般健常人に比し、なぜ高いのか明らかにできなかった。今回の検討から輸血の回数が増加するに伴い、抗 ATLA 抗体陽性率の上昇が明らかになった。こ

の事から HTLV-1 の輸血による感染が示唆され、特に鹿児島県地方などの HTLV-1 浸淫地では、HTLV-1 の輸血による感染を防ぐため早急の対策が必要と思われた。

Introduction

Adult T-cell leukemia (ATL) is a new clinical entity, initially proposed by Takatsuki and his colleagues¹⁾. ATL presents some characteristic clinical and hematological features, and a specific geographical distribution as follows; (1) onset in adulthood; (2) subacute or chronic leukemia with rapidly progressive terminal course; (3) resistance to treatment with current antileukemic agents; (4) leukemic cells are pleomorphic in appearance with deeply indented or lobulated nuclei morphologically, and have peripheral T-cell surface markers; (5) frequent accompaniment of lymphadenopathy, hepatosplenomegaly, skin lesions, pulmonary lesions and hypercalcemia; (6) absence of mediastinal tumor; (7) the birthplaces of patients are clustered in restricted areas of southwestern Japan, including the Kagoshima district²⁻⁴⁾.

A human type-C retrovirus, designated ATL virus (ATLV)/human T-cell leukemia virus type 1 (HTLV-1) has been revealed to be closely related to outbreaks of ATL, etiologically^{5,6)}. That is, antibodies to ATL-associated antigen (ATLA), abbreviated as anti-ATLA, have been detected in the sera of almost all patients with ATL. Furthermore, the incidence of seropositivity has been found to be high in healthy residents in ATL-endemic areas, including members of patients families, but to be very low in healthy residents in non-endemic areas^{4,7)}. HTLV-1 is known to be more transmissible by cell-to-cell rather than free virus-to-cell contact at the tissue culture level^{8,9)}. On the other hand, a subject having anti-ATLA is currently considered to be an HTLV-1 carrier¹⁰⁻¹²⁾. Besides, Okochi et al¹³⁾., Shimoyama et al¹⁴⁾., Hino et al¹⁵⁾., and Fujishita¹⁶⁾ and Momita¹⁷⁾ have reported evidence of seroconversion of blood recipients and have suggested that infection of HTLV-1 occurs via transfused blood cells rather than the plasma component. From these facts, blood, especially cell component transfusion, has become a great problem in ATL-endemic areas, such as the Kagoshima district.

In order to clarify the effect of blood transfusions on the spread of HTLV-1 in the Kagoshima district, we

studied the sera of patients with chronic renal failure (CRF) under hemodialysis, who are more likely to receive massive blood transfusion, for the presence of anti-ATLA.

Materials and methods

Patients with CRF under hemodialysis: Of 234 patients either ambulatory or hospitalized in our related hospitals, Kagoshima district, 115 were male and 119 were female, with an average age of 49.3 ± 14.0 (range 21 to 77) years. Almost all of the patients were born and had lived in the Kagoshima district since the early period of their lives. The seropositivity of the anti-ATLA of the patients was studied in relation to sex, age, duration of hemodialysis and the number of times they had received blood transfusion, in comparison with healthy residents in the Kagoshima district (previously reported)¹⁸⁾.

Immunoassay for anti-ATLA: The method of determining antibodies in this study was by enzyme-linked-immunosorbent assay (ELISA). The assay kit (E-0733) was kindly supplied by Eisai Co., Tokyo. The procedures were as follows. One hundred microliters of normal rabbit serum (NRS) containing 20 mM EDTA buffered to pH 8.0 with 0.05 M Tris-HCL was added to the wells, and coated with ATLA extracted from MT-2 cells which produce large amounts of HTLV-1. Then the test sera (20 μ l) were added at 2°C. One hundred and twenty microliters of NRS was added to the wells as a blank. For the first reaction, the wells were incubated at 40°C for 30 min, and then cooled rapidly to 2°C. After 3 washes with saline, for the second reaction, 100 μ l of alkaline phosphatase-labeled anti-human IgG rabbit serum was added to the wells. After incubation at 40°C for 30 min, the wells were cooled to 2°C and washed 3 times with saline. The final enzyme reaction was carried out by adding 100 μ l of p-nitrophenyl phosphate solution and incubating the mixture at 40°C for 30 min. The reaction was stopped by adding 100 μ l of 4% NaOH after cooling to 2°C. The absorbance at 405 nm was employed using a spectrophotometer (UVIDEC-40, Nihon Bunko Medical). The cut-off value was determined as the optical density rate of the blank plus 0.05.

Sera showing an optical density of 0.30 or higher were taken as positive for anti-ATLA. Moreover, in order to exclude non-specific reactions due to non-ATLA factors, an inhibition test was carried out for those sera showing an optical density from the cut-off value to 0.30. After the test sera (20 μ l) had been added to the non-coated wells of ATLA, NRS (100 μ l) as a control and ATLA solution (100 μ l) for inhibition were added to each well. After incubation at 37°C for 60 min, the wells were cooled to 2°C, and the sera of the wells were then transferred to the ATLA-coated wells and the reaction was carried out by the same procedures as those mentioned above. Sera showing an inhibition of above 50% were regarded as being seropositive.

Results

1. The incidence of anti-ATLA in patients with CRF under hemodialysis

Thirty-two out of 115 males (27.8%), 47 out of 119 females (39.5%) and 79 out of the total of 234 (33.8%) were found to be seropositive. The anti-ATLA ser-

	positive cases of anti-ATLA	anti-ATLA positivity (%)
hemodialysis patients (male)	32	27.8 (32 cases/115 cases)
hemodialysis patients (female)	47	39.5 (47/119)
total	79	33.8 (79/234)
healthy residents (Kagoshima district) 16 y~86 y	297	11.7 (297/2,533)
blood donors (Kagoshima district) 16 y~64 y	41	6.9 (41/596)

Table 1 The incidence of anti-ATLA in patients with CRF under hemodialysis

opositivity of the patients was statistically higher than that of normal residents (297 out of 2,533, 11.7%) and blood donors (41 out of 596, 6.9%) in the Kagoshima district ($p < 0.01$)¹⁸⁾. The anti-ATLA reactivity in females was higher than that in males, but no statistical difference was recognized between the sexes (Table 1).

2. Correlation between the age of the patients and anti-ATLA positivity

The patients were divided into 6 groups according to each ten-years age period. The positive rate of anti-ATLA increased with age in the normal healthy residents in Kagoshima, while it did not increase in the patients. Comparing the seropositivity of the patients with that of normal healthy residents in the Kagoshima district, the former was statistically higher in every age group ($p < 0.01$) (Table 2).

3. Correlation between the duration of hemodialysis and anti-ATLA positivity

The patients were divided into four groups according to the duration of hemodialysis, namely, Group I ; less than one year (35 cases), Group 2 ; from one year to less than 5 years (134 cases), Group 3 ; from 5 years to less than 10 years (52 cases), and Group 4 ; from 10 years to 13 years (11 cases). The seropositivity in each of the groups was 28.6 % (10 out of 35), 34.3% (46 out of 134), 34.6% (18 out of 52) and 45.5% (5 out of 11), respectively (Table 3).

4. Correlation between episodes of blood transfusion and the incidence of anti-ATLA

The blood units transfused were mainly whole blood (fresh or stored) and packed red cells supplied by Kagoshima Red Cross Blood Center. The positive rate of anti-ATLA was 37.7% (61 out of 162) in group A with episodes of blood transfusion and 25.0% (18 out of 72) in group B without episodes of blood transfu-

	21~30 y	31~40 y	41~50 y	51~60 y	61~70 y	71~79 y
positive cases of anti-ATLA	6	18	13	18	16	8
anti-ATLA positivity (%)	28.5 (6/21 cases)	31.6 (18/57)	28.3 (13/46)	37.5 (18/48)	33.3 (16/48)	57.1 (8/14)
anti-ATLA positivity of healthy residents (Kagoshima district) (%)	6.5 (21/321)	8.1 (26/320)	11.4 (63/553)	12.9 (84/649)	17.5 (65/371)	25.2 (29/115)

Table 2 Correlation between the age of hemodialysis patients and anti-ATLA positivity

sion. The positive rate of anti-ATLA in both the two groups was higher than that in the healthy residents in the Kagoshima district ($p < 0.01$). No statistical differences between group A and group B were found with regard to the incidence of anti-ATLA (Table 4).

5. Correlation between the number of blood transfusions received and anti-ATLA positivity

The patients were divided into 3 groups, the first group without blood transfusions (72 cases), the second group with from 1 to 10 blood transfusions (140

cases) and the third group with from 11 to 79 transfusions (22 cases). The seropositivity of each group was 25.0% (18 out of 72), 34.3% (48 out of 140) and 59.1% (13 out of 22), respectively. No differences in anti-ATLA positivity were seen between the group with blood transfusion and the group without blood transfusion, as mentioned above. However, the incidence of anti-ATLA increased with the number of blood transfusions given ($p < 0.05$) (Table 5).

6. Correlation between anti-ATLA positivity, age and the episodes of blood transfusion

In patients without any episode of blood transfusion, the seropositivity was similar in each of the age groups and was not increased with age except for a small number of patients over 71 years old. The same findings were also seen in patients with episodes of blood transfusion (Table 6).

Discussion

From this study of 234 patients with CRF under hemodialysis, anti-ATLA was detected by ELISA in 32 out of 115 (27.8%) males, 47 out of 119 (39.5%) females and 79 out of a total of 234 (33.8%). The seropositivity of the patients was statistically higher than that of the normal residents in the Kagoshima district (297 out of 2,533, 11.7%)¹⁸⁾. Sixty-one out of 79 patients with anti-ATLA had had episodes of blood transfusion. The incidence of anti-ATLA in patients with blood transfusion increased with the number of blood transfusions given. The results of our study thus suggested the possibility of transmission of HTLV-1 by blood transfusion, as others have reported¹³⁻¹⁷⁾.

Interestingly, the seropositivity of patients without blood transfusion (18 cases) was also higher than that of healthy residents in the Kagoshima district. Possible explanations for the increased appearance of the antibody in these patients are as follows: (1) some relation between HTLV-1 and the outbreak of renal failure, (2) chance clustering of HTLV-1 in these

duration of hemodialysis	positive cases of anti-ATLA	anti-ATLA positivity (%)
less than 1 year	10	28.6 (10/35 cases)
more than 1 year but less than 5	46	34.3 (46/134)
more than 5 years but less than 10	18	34.6 (18/52)
from 10 to 13 years	5	45.5 (5/11)
total	79	33.8 (79/234)

Table 3 Correlation between the duration of hemodialysis and anti-ATLA positivity

episodes of blood transfusion	positive cases of anti-ATLA	anti-ATLA positivity (%)
(+)	61	37.7 (61/162 cases)
(-)	18	25.0 (18/72)
total	79	33.8 (79/234)

Table 4 Correlation between episodes of blood transfusion and anti-ATLA positivity

number of blood transfusions	anti-ATLA positive cases	anti-ATLA positivity (%)
0	18	25.0 (18/72 cases)
1~10	48	34.3 (48/140)
11~79	13	59.1 (13/22)
total	79	33.8 (79/234)

Table 5 Correlation between the number of blood transfusions and anti-ATLA positivity

	21~30 y	31~40 y	41~50 y	51~60 y	61~70 y	71~79 y	total
without blood transfusion	25.0% (2/8 cases)	15.4 (2/13)	25.0 (3/12)	20.0 (3/15)	29.4 (5/17)	42.9 (3/7)	25.0 (18/72)
with blood transfusion	30.8% (4/13 cases)	36.4 (16/44)	29.4 (10/34)	45.5 (15/33)	35.5 (11/31)	71.4 (5/7)	37.7 (61/162)
healthy residents (Kagoshima district)	6.5% (21/321 persons)	8.1 (26/320)	11.4 (63/553)	12.9 (84/649)	17.5 (65/371)	25.2 (29/115)	12.4 (288/2,329)

Table 6 Correlation between anti-ATLA positivity, age and episodes of blood transfusion

patients families, (3) increased production of anti-ATLA in these patients, (4) transmission of HTLV-1 from medical or paramedical co-workers in hemodialysis units, (5) transmission of HTLV-1 due to the techniques used in hemodialysis. The first possibility is unlikely, since patients with CRF are not especially clustered in ATL-endemic areas. However, some renal diseases are caused by immune mechanisms, such as the deposition of immune complexes in renal glomeruli^{19,20}. It might therefore be important to study the correlation between ATLA-anti-ATLA complexes and renal failure. The second possibility is also unlikely but cannot be completely excluded, since HTLV-1 is frequently clustered in the family of the seropositive person^{12,18,21,22}. It is suggested that the transmission of HTLV-1 can occur from mother to child transplacentally or in early infancy, and also between spouses, especially from husband to wife^{12,18,21,22}. The third possibility also seems unlikely, since the production of anti-HBs antibody has been reported to be decreased in hemodialysis patients after HBs antigen vaccination^{23,24}. The fourth possibility is also unlikely, as the anti-ATLA reactivity of hemodialysis co-workers in our hospitals was similar to that of healthy residents in the Kagoshima district (unpublished data). The fifth possibility was further unlikely, since the dialysers used in our hospitals are disposed of and anti-ATLA reactivity was revealed to be unrelated to the duration of hemodialysis in this study.

Consequently, it appears to be conclusive that HTLV-1 is transmissible by blood transfusion, so that blood transfusion must be considered to be an important pathway of HTLV-1 infection. In this study, we were unable to clarify the reason why the anti-ATLA of patients without blood transfusion was statistically higher than that of the healthy residents in Kagoshima¹⁸. In this respect, a further study may be necessary in future. As it is suspected that there are many blood units harboring HTLV-1 in Kagoshima¹⁸, the number of carriers of the virus may be increased through the use of blood transfusion and these individuals in turn could become sources of further infection. Although no outbreak of ATL has yet been linked to blood transfusion, it seems necessary to carry out mass-screening of blood units as soon as possible to

prevent the expansion of HTLV-1 infection in ATL-endemic areas such as the Kagoshima district.

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