シンポジウム7「HTLV-1感染予防」

HTLV-1母子感染予防のための乳汁栄養の選択とその問題点

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コホート研究

ヒトT細胞白血病ウイルス（human T cell leukemia virus type 1，以下HTLV-1）は，成人T細胞白血病（adult T cell leukemia，以下ATL）やHTLV-1関連脊髄症（HTLV-1 associated myelopathy，以下HAM）などの原因ウイルスとして知られている．ATLは母子感染によって長い年月を経て発症する（中央値67歳）死亡率の高い難治性の疾患であり，生涯発症率はキャリアの5%である．また，難病に指定されているHAMは30～50歳代に発症することが多く，母子感染でも水平感染でも発症するが，ATLに比べキャリアの生涯発症率は低く0.3%である．現時点では使用可能なワクチンは開発されておらず，唯一有効な感染予防法は，HTLV-1により感染したTリンパ球（$CD4^+$）が体内に侵入することを防ぐほかない．

母子感染経路として重要なのが経母乳感染で，HTLV-1キャリアの母親が3カ月以上母乳を与えると母子感染率は約20%となる．だが，出生直後から完全に母乳栄養を遮断し，人工栄養を行ったとしても約3%の児が感染する（この感染経路については不明な点が多い）．人工栄養以外の母子感染予防法としては，90日以内の短期母乳栄養および冷凍母乳栄養がある（表1[1]）．短期母乳の感染予防効果は，母体からの移行抗体が母乳を介したウイルスの侵入をブロックすることや，感染細胞の暴露が短期間であることに由来すると推測される．冷凍母乳は感染したTリンパ球が冷凍により破壊されることが予防効果をもたらすと考えられている．-20℃以下の家庭用冷凍庫で24時間以上冷凍後に溶解して与えることができるが，“食品の細胞を壊さずおいしく食べられる”とうたっているcell alive system（CAS）が用いられている冷凍庫では，感染細胞が破壊されにくいため利用することができない[2]．それぞれの乳汁栄養法には表2に示したような利点や課題があり，人工乳，短期母乳，冷凍母乳のどれをとっても完全に母子感染を予防できる訳ではない．とくに留意する必要があるのは短期母乳栄養で，乳汁分泌が順調になってきた時期に母乳を中止することになるため，ときに3カ月を超えて母乳を与え続けてしまうことになりかねない．この場合，母子感染のリスクが増加することが懸念される．

人工栄養による母子感染予防法は症例の集積が多く，確立された手段として位置づけられている．一方，短期母乳や冷凍母乳については検討された症例数も少

表1　各種乳汁栄養法とHTLV-1母子感染率

乳汁栄養法	検討対象数	陽性者数	陽性率（%）	機序
母乳栄養（90日以上）	525	93	17.7	・中和抗体の減少 ・感染細胞の暴露が長期間である
人工栄養	1,553	51	3.3	・感染細胞の暴露がない
短期母乳栄養（90日未満）	162	3	1.9	・中和抗体の存在 ・感染細胞の暴露が短期間である
冷凍母乳	64	2	3.1	・感染細胞の破壊・死滅

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表2　HTLV-1母子感染予防のために利用されている乳汁栄養法の利点と課題

	完全人工栄養	短期母乳栄養	冷凍母乳栄養
利点	・最も確実な母子感染予防法である	・ある程度母乳栄養の利点が得られる ・短期間であるが直接授乳（直母）が可能である	・ある程度母乳栄養の利点が得られる（とくに早産・低出生体重児）
課題	・母親の満足が得られにくい？ ・母乳栄養の利点が得られない ・コストがかかる ・生活習慣病のリスクが増加する？	・現時点では十分なエビデンスがない ・母乳を遮断することがときに困難となるため，十分な支援が必要である ・母乳分泌を抑制するための薬剤服用が必要	・現時点では十分なエビデンスがない ・母乳パックなどの諸費用がかかる ・手間がかかる ・cell alive system（CAS）の冷凍庫は使用できない ・直接授乳（直母）ができない

なく，十分なエビデンスが確立されているわけではない．また，最近の我が国における母乳哺育の普及により，多くの母親が母乳栄養を希望していることや，人工栄養が将来のメタボリックシンドロームのリスクを増加させるかもしれないという懸念に加え，人工栄養でも完全に母子感染を防ぐことができないという事実は，母子感染予防のための乳汁を選択するにあたり，母親の意志決定をより複雑なものにしている．その他，妊婦健診においてHTLV-1抗体スクリーニング検査陽性者は，確認検査としてWB法が行われることになっているが，そのうち10～20%が判定保留となっている．しかし，現状では判定保留者からの母子感染率やその予防方法についても明らかとなっていない[3]．

そこで，母子感染予防と児の健全な育成の視点に立ち，確認検査でHTLV-1抗体陽性あるいは判定保留妊婦から出生した児の適切な乳汁栄養法を明らかにするとともに，将来の感染者を確実に減少させることを目的として，厚生労働科学研究班「HTLV-1母子感染予防に関する研究：HTLV-1抗体陽性妊婦からの出生児のコホート研究」（研究代表者：板橋家頭夫）が立ち上げられた[3]．現時点では結論は得られていないが，コホート研究の成果に期待が寄せられている．

（本稿の一部は，厚生労働科学研究「HTLV-1母子感染予防に関する研究：HTLV-1抗体陽性妊婦からの出生児のコホート研究」により行われたものである）

文　献

1) 斎藤滋：厚生労働省科学研究費補助金・特別研究事業「HTLV-1の母子感染予防に関する研究」．平成21年度総括・分担報告書．www.mhlw.go.jp/bunya/kodomo/boshi-hoken16/dl/04.pdf （医師向け手引き）
2) 森内浩幸：厚生労働科学研究費補助金・特別研究事業「ヒトT細胞白血病ウイルス-1型（HTLV-1）母子感染予防のための保健指導に関する標準化に関する研究」．平成22年度研究報告書．www.mhlw.go.jp/bunya/kodomo/boshi-hoken16/dl/05.pdf（保健指導マニュアル）
3) 板橋家頭夫：厚生労働科学研究補助金（成育疾患克服等次世代育成基盤研究事業）「HTLV-1母子感染予防に関する研究：HTLV-1抗体陽性妊婦からの出生児のコホート研究」平成23-25年度総括研究報告書．

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Standardization of Quantitative PCR for Human T-Cell Leukemia Virus Type 1 in Japan: a Collaborative Study

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Quantitative PCR (qPCR) analysis of human T-cell leukemia virus type 1 (HTLV-1) was used to assess the amount of HTLV-1 provirus DNA integrated into the genomic DNA of host blood cells. Accumulating evidence indicates that a high proviral load is one of the risk factors for the development of adult T-cell leukemia/lymphoma and HTLV-1-associated myelopathy/tropical spastic paraparesis. However, interlaboratory variability in qPCR results makes it difficult to assess the differences in reported proviral loads between laboratories. To remedy this situation, we attempted to minimize discrepancies between laboratories through standardization of HTLV-1 qPCR in a collaborative study. TL-Om1 cells that harbor the HTLV-1 provirus were serially diluted with peripheral blood mononuclear cells to prepare a candidate standard. By statistically evaluating the proviral loads of the standard and those determined using in-house qPCR methods at each laboratory, we determined the relative ratios of the measured values in the laboratories to the theoretical values of the TL-Om1 standard. The relative ratios of the laboratories ranged from 0.84 to 4.45. Next, we corrected the proviral loads of the clinical samples from HTLV-1 carriers using the relative ratio. As expected, the overall differences between the laboratories were reduced by half, from 7.4-fold to 3.8-fold on average, after applying the correction. HTLV-1 qPCR can be standardized using TL-Om1 cells as a standard and by determining the relative ratio of the measured to the theoretical standard values in each laboratory.

Human T-cell leukemia virus type 1 (HTLV-1) is present in certain regions of endemicity, including sub-Saharan Africa, the Caribbean, parts of South America, the Middle East, Melanesia, and southwest Japan (1, 2). HTLV-1 mainly infects vertically from infected mothers to children through breast-feeding and horizontally between adults by sexual intercourse and transmission through transfusions with blood products. Although the majority of infected people live without any symptoms, some HTLV-1 carriers suffer from adult T-cell leukemia (ATL), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and HTLV-1 uveitis/HTLV-1-associated uveitis after a long period of latency (3).

HTLV-1 mainly infects CD4-positive peripheral blood cells, and the provirus is integrated into the host genome. Generally, HTLV-1 infection is determined by serological testing. Detection of proviral DNA in peripheral blood mononuclear cells (PBMCs) by PCR is one of the methods to detect the infection. Quantitation of both the provirus and a cellular gene in PBMCs by TaqMan quantitative PCR (qPCR) enables calculation of the percentages of

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infected cells in the peripheral blood (proviral load [PVL] [copies per 100 cells]). Accumulating evidence shows that a high PVL is a risk factor for the development of ATL and HAM/TSP. Therefore, it is expected that HTLV-1 qPCR can be an effective tool to assess the risk for development of these diseases (4, 5).

Several in-house qPCR methods to quantitate the PVL are used in many laboratories in Japan. However, the materials for the qPCR standard curve and primers for the HTLV-1 provirus and cellular control genes vary among laboratories. There are at least five cellular internal control (IC) genes used in Japanese laboratories (albumin gene, β-actin gene, β-globin gene, CD81 gene, recombination-activating gene 1, and RNase P gene). These conditions give rise to difficulty in direct comparison of PVLs across laboratories. For example, when the PVL was analyzed in the same samples among laboratories in Japan, there were up to 5-fold differences between the laboratories (6). For this reason, standardization of HTLV-1 qPCR is required to predict the risk for development of HTLV-1-associated diseases correctly.

WHO international standards (ISs) of nucleic acid amplification techniques (NATs) have been established for human immunodeficiency virus type 1, hepatitis B virus, and hepatitis C virus, and positive plasma samples that have been assigned international units for these viruses are available (7–9). However, a defined IS has not been established for HTLV-1 NATs. Because the target material of HTLV-1 qPCR is genomic DNA obtained from cells, we considered that a specific cell type would be a desirable material for the HTLV-1 qPCR standard.

Previously, we found that the HTLV-1 provirus copy number in TL-Om1 cells, an ATL cell line, is 1.8 copies/cell, and its karyotype is polyploidy of 4N (10). These precise genomic properties are useful for TL-Om1 cells to be a candidate material for the HTLV-1 qPCR standard.

In this study, we hypothesized that standardization of HTLV-1 qPCR could be achieved by using TL-Om1 cells as a standard material for HTLV-1 qPCR. To minimize the differences between laboratories, we tried to correct PVLs by adjusting the results of laboratories to absolute values of the standard prepared with the TL-Om1 cell line. A collaborative study was conducted with the participation of eight laboratories that perform HTLV-1 qPCR routinely in Japan.

MATERIALS AND METHODS

Plasmid, cells, and cell culture. The plasmid used in this study (pUC-HTLV-1) has been reported previously (6). In brief, pUC-HTLV-1 was prepared by ligating a SacI-digested fragment of HTLV-1 (equivalent to the region from nucleotide [nt] 503 to 8780 of ATK-1; NCBI accession number J02029) into the SacI cloning site of pUC18.

TL-Om1 cells were a kind gift from Kazuo Sugamura (Miyagi Cancer Center Research Institute) (11). Jurkat clone E6-1 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). PBMCs were purchased from AllCells Inc. (Alameda, CA, USA). TL-Om1 cells were maintained in RPMI 1640 (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA), 2 mmol/liter L-glutamine, and 10 ng/ml interleukin-2 (PeproTech, London, United Kingdom). Jurkat cells were maintained in RPMI 1640 supplemented with 10% FBS containing 100 U/ml penicillin-streptomycin and 2 mmol/liter L-glutamine. Cryopreserved PBMCs were resuspended in RPMI 1640 supplemented with 10% FBS at 37°C according to the protocol provided by AllCells Inc.

Clinical samples and extraction of genomic DNA. The study was approved by the ethical review boards at the National Institute of Infectious Diseases (IRB approval no. 392). HTLV-1-positive peripheral blood samples were obtained from healthy HTLV-1 carriers registered in the Joint Study on Predisposing Factors of ATL Development (JSPFAD) as follows. PBMCs were separated from the blood samples by Percoll density gradient centrifugation at the National Institute of Infectious Diseases. The PBMCs were resuspended in Cellbanker (TaKaRa Bio, Osaka, Japan) at 1×10^6 cells per 100 μl and frozen at lower than −80°C. Frozen PBMCs packed in dry ice were then provided to the laboratories by the National Institute of Infectious Diseases. Genomic DNA was extracted using QIAamp blood DNA mini-, midi-, or maxikits (Qiagen, Hilden, Germany) according to the manufacturer's instructions or by SDS-proteinase K digestion and phenol-chloroform extraction followed by ethanol precipitation. The concentration of purified DNA was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Yokohama, Japan) or a Qubit fluorometric quantitation kit (Life Technologies, Tokyo, Japan).

Preparation of HTLV-1 qPCR standards. To evaluate candidate materials for HTLV-1 qPCR standardization, we prepared TL-Om1 cells diluted with PBMCs at 20, 4, 0.8, and 0.16% and TL-Om1 cells diluted with Jurkat cells at 10, 2, 0.4, and 0.08%. TL-Om1 cells diluted with PBMCs were prepared at laboratory B, and TL-Om1 cells diluted with Jurkat cells were prepared at laboratory A. TL-Om1 cells, PBMCs, and Jurkat cells suspended in Cellbanker at 1×10^7 cells/ml were serially diluted at each of the indicated concentrations. The cells were then resuspended at 1×10^6 cells per 100 μl and frozen at lower than −80°C until use.

To confirm whether TL-Om1 cells were diluted correctly, a serial dilution of TL-Om1 cells with uninfected PBMCs was prepared as follows. TL-Om1 cells were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Sigma) according to the manufacturer's protocol. Briefly, TL-Om1 cells suspended at 1×10^6cells/ml in 0.1% bovine serum albumin–phosphate-buffered saline were stained with CFSE at a final concentration of 1 μM and then incubated for 10 min at 37°C. The cells were resuspended in a 5-fold volume of RPMI 1640 supplemented with 10% FBS and incubated at 4°C for 5 min. The cells were then washed three times with RPMI 1640 supplemented with 10% FBS at 4°C. Stained TL-Om1 cells were cultured for 3 days and then harvested. TL-Om1 cells and PBMCs were washed twice with RPMI 1640 supplemented with 10% FBS at 4°C. The TL-Om1 cells were serially diluted with PBMCs at 20, 4, 0.8, and 0.16%. The concentrations of TL-Om1 cells were calculated from the percentages of CFSE-positive cells analyzed at the National Institute of Infectious Diseases using flow cytometry with a JSAN flow cytometer (Bay Bioscience, Kobe, Japan). The plasmid (pUC-HTLV-1) used as a reference was prepared by diluting with purified genomic DNA from PBMCs.

qPCR and correction of PVLs. Eight Japanese laboratories (one national institute laboratory, five university laboratories, one Japanese Red Cross laboratory, and one diagnostic test company laboratory) participated in this study. The protocols for HTLV-1 qPCR performed in the eight laboratories have been reported previously (5, 12–18) (see Table S1 in the supplemental material). To evaluate candidate materials for the HTLV-1 standard, the laboratories extracted DNA from the panel of dilutions and measured the PVL once. To standardize HTLV-1 qPCR, PVLs (copies per 100 cells) were measured with purified DNA in laboratories independently three times on different days. To evaluate all steps of the preparation, each measurement began with extraction of genomic DNA from new frozen samples, and testing was performed once with the extracted DNA. The analyses were performed in 2011 and again in 2013. Results were statistically analyzed at the National Institute of Infectious Diseases with the parallel-line assay (PLA), and relative ratios to the theoretical values of PVLs in TL-Om1 cell dilutions were calculated for each laboratory (19). The PLA is a statistical method used to evaluate the potency of international or national standards for biologics. The PVLs measured in each laboratory were then corrected based on the relative ratio. Correction of clinical PVLs was made by dividing the measured PVLs by the relative ratio of each laboratory.

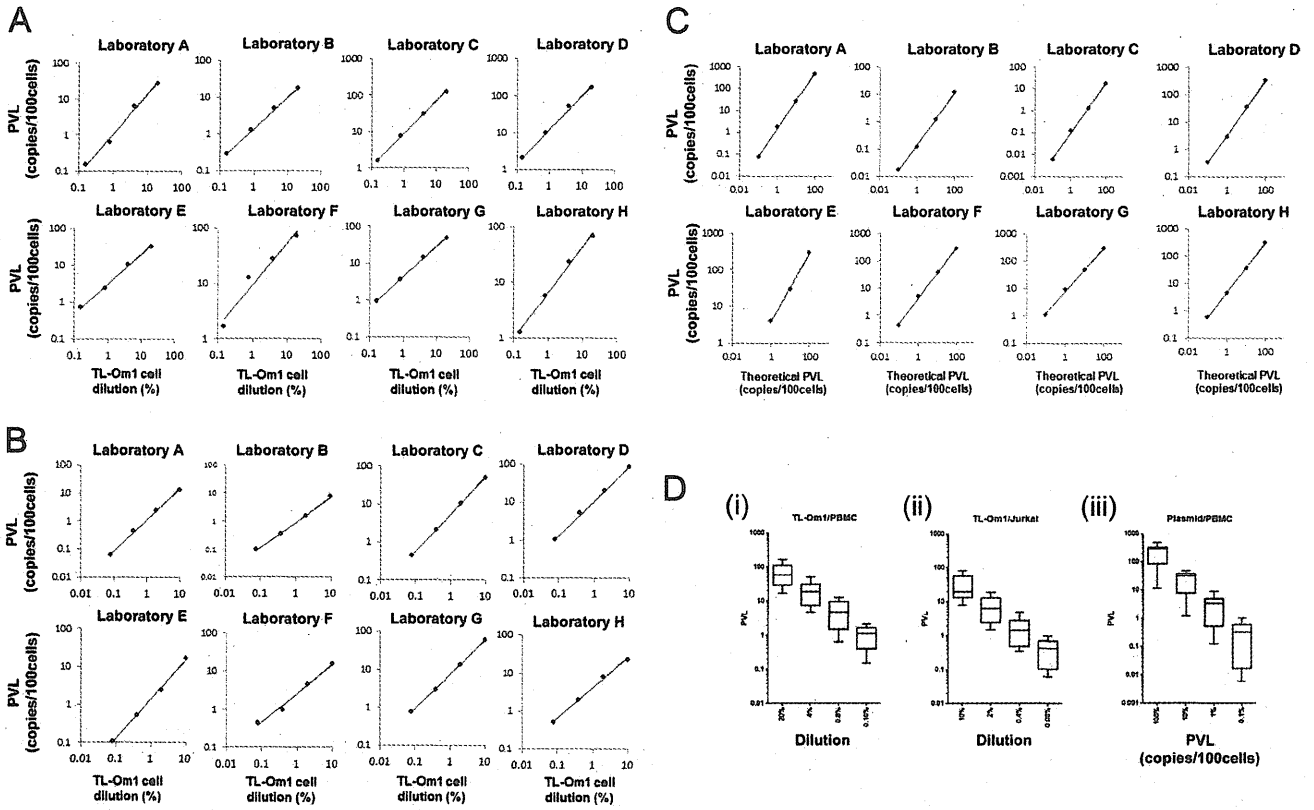

FIG 1 Evaluation of TL-Om1 cell dilution panels for HTLV-1 qPCR standardization. (A to C) Proviral loads (PVLs) (copies/100 cells) of candidate standards for HTLV-1 qPCR were measured by eight Japanese laboratories (laboratories A to H) as TL-Om1 cell dilutions with PBMCs (A), TL-Om1 cell dilutions with Jurkat cells (B), and plasmid DNA dilutions with PBMC genomic DNA (C). Results of each dilution are plotted in the log-log graphs. The horizontal axis shows the percent dilution of TL-Om1 cells. The vertical axis shows the measured values in each laboratory. Lines show evaluation of the linear correlation between TL-Om1 cell dilutions (percent) and measured values (PVL, copies/100 cells) in each laboratory. (D) Distribution of results from laboratories using the candidate standards. Data are shown as box plots. The horizontal bar in the box of the graph shows the median. Error bars show the maximum to minimum scores. (i) TL-Om1 cells diluted with PBMCs; (ii) TL-Om1 cells diluted with Jurkat cells; (iii) HTLV-1 plasmid DNA diluted with PBMC genomic DNA. For detailed statistical data, see Table S3 in the supplemental material.

RESULTS

Evaluation of candidate materials for HTLV-1 qPCR standards. For standardization of HTLV-1 qPCR, we tried to fit measured values from the various laboratories to the theoretical values of the standard. In addition, to avoid differences because of specific concentrations, we analyzed the measured values at several concentrations of diluted cells.

To determine appropriate candidate materials for standardization of HTLV-1 q-PCR, we prepared three different types of materials. Two sets were prepared from TL-Om1 cells diluted with PBMCs (TL-Om1 cell concentrations of 0.16, 0.8, 4, and 20%) or Jurkat cells (TL-Om1 concentrations of 0.08, 0.4, 2, and 10%), and the other set was a plasmid diluted with genomic DNA from human PBMCs (0.1, 1, 10, and 100 copies per cells). Genomic DNA was extracted from these samples, and the PVL was measured by the eight laboratories with their in-house qPCR methods (see Table S1 in the supplemental material). All three candidate materials showed linear correlations between the concentration of the standard material and the measured values in a dose-dependent manner in all laboratories (Fig. 1A to C; see Table S2 in the supplemental material).

By focusing on each dilution, the PVLs obtained from measuring the plasmid dilutions showed a larger variation between laboratories at all concentrations (differences ranged from 39- to 173-fold) than those from the other two cell dilution panels (9.5- to 19.7-fold for TL-Om1 cells/PBMCs and 10.5- to 15.7-fold for TL-Om1 cells/Jurkat cells) (Fig. 1D; see Table S3 in the supplemental material).

Based on these results, we found that TL-Om1 cells diluted with PBMCs or Jurkat cells are suitable standard materials, because the use of these standards by the laboratories to measure PVLs was more accurate than use of the plasmid candidate.

Preparation and determination of PVLs in cell dilution standards. Because the karyotype of PBMCs is strictly 2N, we considered that PBMCs would be more appropriate than Jurkat cells to prepare the standard in this study. Therefore, to normalize the results of HTLV-1 qPCR obtained by different methods and laboratories, we prepared a panel of TL-Om1 cell dilutions. The concentrations for the standard were 0.16, 0.8, 4, and 20% TL-Om1 cells in PBMCs. These concentrations were chosen to cover the range of PVLs that were observed in HTLV-1 carriers (4). PVLs of these TL-Om1 cell dilutions were calculated from both the percentage of TL-Om1 cells in PBMCs, which was analyzed by flow cytometry, and the copy number of HTLV-1 provirus and karyotype of TL-Om1 cells as re-

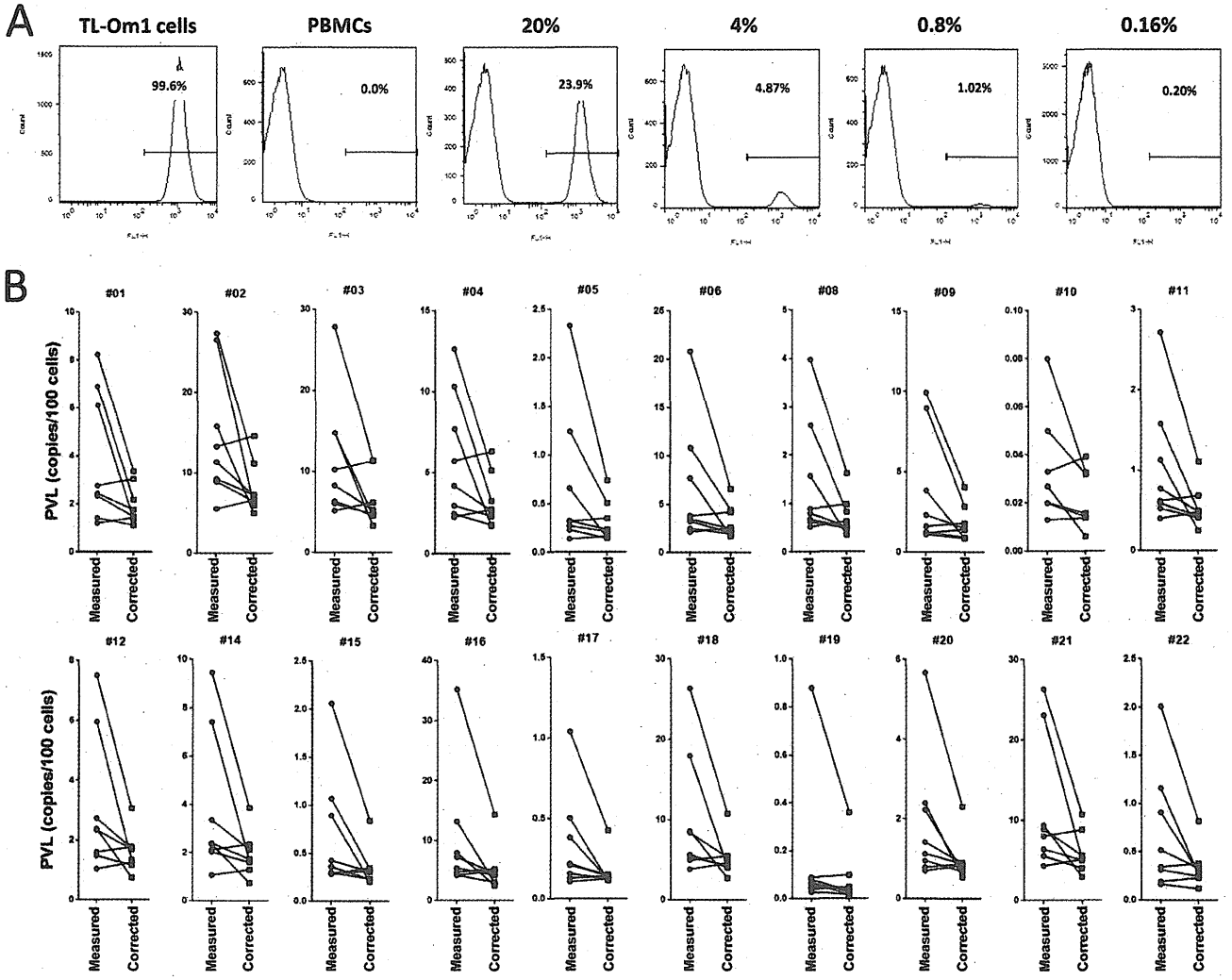

FIG 2 Standardization of HTLV-1 qPCR with standard materials prepared from cells. (A) Analysis of the concentrations of TL-Om1 cells diluted with uninfected PBMCs by flow cytometry. TL-Om1 cells stained with CFSE were diluted with unstained PBMCs at 20, 4, 0.8, and 0.16%. The percentage of CFSE-positive cells was defined as the concentration of TL-Om1 cells. (B) PBMCs from HTLV-1 carriers (registered in JSPFAD) were analyzed for their PVLs by the eight laboratories. Panels show the PVLs (copies/100 cells) of measured values and corrected values. Corrections were made by dividing measured values by the relative ratio for each laboratory, which is shown in Table 1. Among 22 samples, two samples (07 and 13) were eliminated from the analyses because they had negative qPCR results in several laboratories. In addition, the PVLs of these two samples measured in other laboratories were extremely low.

ported previously (10) (Fig. 2A and Table 1). These prepared aliquots of cells were frozen and provided to the eight laboratories.

Standardization of HTLV-1 qPCR using TL-Om1 cell dilutions. First, we evaluated differences in the laboratories against the theoretical values of the TL-Om1 cell dilutions. Genomic DNA of these TL-Om1 cell dilutions was extracted in each laboratory, and the PVL of each dilution was measured by in-house qPCR methods.

To assess accuracy, the panel of TL-Om1 cell dilutions was measured three times independently on different days to determine the day-to-day variation. Additionally, to assess reproducibility, these procedures were performed twice across years. The results were statistically analyzed with the PLA. As shown in Table 2, the relative ratio of the value obtained in each laboratory to the theoretical value was calculated. The majority of laboratories had a small range of 95% confidence intervals, indicating that the laboratories could measure the PVL accurately. The relative ratio for each laboratory varied from 0.84 to 4.45 in 2011. A maximal 5.3-fold difference was observed between laboratories. In addition, a maximal 2.8-fold difference was observed in 2013. The relative ratios for each laboratory measured in 2011 and 2013 were similar, indicating high reproducibility over time.

TABLE 1 Calculation of theoretical values of the TL-Om1 cell dilutions[a]

Sample name	% of TL-Om1 cells	Copies/100 cells		Theoretical PVL
		HTLV-1 gene	IC gene	
20%	23.9	43.5	247.8	35.1
4%	4.87	8.86	209.7	8.45
0.8%	1.02	1.86	202.0	1.84
0.16%	0.20	0.37	200.4	0.37

[a] Calculations were performed by setting the HTLV-1 gene copy number in TL-Om1 cells as 1.8 copies/cell, the IC (RNase P) gene copy number in TL-Om1 cells as 4 copies/cell, and the IC gene copy number in PBMCs as 2 copies/cell.

TABLE 2 Relative ratios of laboratory values to the theoretical values of the PVLs of TL-Om1 cell dilutions

Laboratory	First study (2011)		Second study (2013)	
	Relative ratio[a]	95% confidence interval	Relative ratio	95% confidence interval
A	1.38	1.13–1.70	1.38	1.09–1.75
B	1.27	1.11–1.46	1.25	1.10–1.41
C	4.45	3.53–5.65	2.94	2.57–3.38
D	0.84	0.72–0.96	1.30	0.91–1.87
E	0.91	0.65–1.26	1.05	0.97–1.13
F	3.16	2.05–5.00	2.35	1.49–3.84
G	2.45	1.90–3.20	1.62	1.27–2.06
H	1.58	1.07–2.35	2.04	1.87–2.23

[a] These values were used for correction in the multicenter study.

Next, we examined the usefulness of the TL-Om1 cell line to standardize HTLV-1 qPCR for clinical samples. Twenty PBMC samples isolated from the blood of healthy HTLV-1 carriers who were registered in JSPFAD (see Materials and Methods) were subjected to DNA extraction in each laboratory. The PVLs in these DNA samples were measured by in-house HTLV-1 qPCR methods in each laboratory. Next, the carrier PVLs were corrected by dividing by the relative ratio of each laboratory. After the correction, as shown in Fig. 2B, the difference between laboratories became apparently smaller for all samples. The average of maximum interlaboratory differences was reduced by half, from 7.4- to 3.8-fold. In addition, the PVLs measured at seven laboratories coincided for some samples (for example, samples 15, 16, 17, 18, and 20) after the correction, indicating that these laboratories would be able to perform testing with high accuracy. These results strongly indicated that HTLV-1 qPCR could be standardized by applying this method.

Evaluation of intralaboratory differences in HTLV-1 qPCR by day-to-day and periodic analysis. Although the results from the majority of laboratories were consistent after the correction, slight differences were still observed in some laboratories. We analyzed the sequences of the qPCR target regions in the HTLV-1 genome in 18 out of 20 carrier samples, but there were no critical mutations that could cause the differences (data not shown). Last, to evaluate and monitor the long-term reproducibility of the test methods, the same samples were measured as a run control by all laboratories for 3 years periodically, followed by evaluation of day-to-day and periodic reproducibility (Fig. 3). Day-to-day variations were 2.6-fold between maximum and minimum values in laboratory F in November 2011, although the day-to-day variations in all laboratories were extremely small throughout the study (Table 3). In addition, similar results were obtained in the evaluation of reproducibility. We compared the means of three independent measurements across four testing periods. Variations of about 2-fold were observed in laboratories C, F, and G, whereas extremely small variations were found throughout the testing periods for the majority of laboratories (Table 3). These results strongly indicated that laboratories could obtain reproducible results with the same samples.

DISCUSSION

As JSPFAD investigators have found that a high PVL is a risk factor for the development of ATL (4), measurement of the PVL by qPCR has become an important test to predict and control the

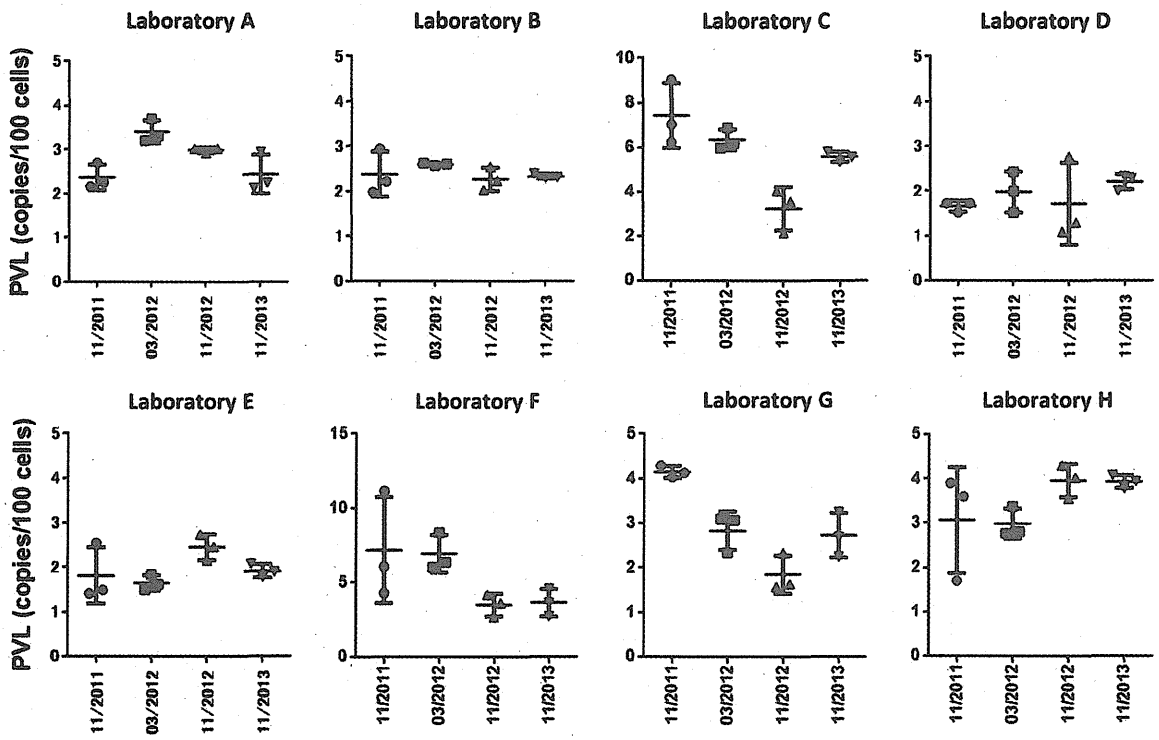

FIG 3 Day-to-day and periodic variations of measured values in the eight laboratories All laboratories measured the PVL of 0.8% TL-Om1 cells in PBMCs as a run control for 3 years. In each testing period, measurements were performed three times independently on different days. Dots show the PVL (copies/100 cells) for each laboratory in each testing period. The bars indicates the averages of the results.

TABLE 3 Day-to-day and periodic variations of measured values in the eight laboratories

Laboratory	Day-to-day variation (ratio)[a] in mo/yr: 11/2011	3/2012	11/2012	11/2013	Periodic variation (ratio)[b]
A	1.25	1.15	1.04	1.38	1.44
B	1.49	1.02	1.26	1.04	1.15
C	1.45	1.15	1.87	1.09	2.29
D	1.14	1.60	2.54	1.16	1.33
E	1.80	1.23	1.27	1.17	1.49
F	2.60	1.39	1.57	1.69	2.05
G	1.06	1.32	1.49	1.44	2.24
H	2.29	1.22	1.21	1.08	1.32

[a] Ratio between maximum and minimum PVLs in a time period.
[b] Ratio between maximum and minimum mean PVLs among four time periods.

TABLE 4 Relative ratios of laboratory to theoretical values determined simply by comparison of the 0.8% TL-Om1 single dilution

Laboratory	First study (2011) Relative ratio	95% confidence interval	Second study (2013) Relative ratio	95% confidence interval
A	1.28	0.95–1.73	1.31	0.85–2.04
B	1.28	0.76–2.13	1.27	1.21–1.35
C	4.00	2.49–6.41	3.01	2.73–3.35
D	0.90	0.75–1.08	1.27	0.98–1.46
E	0.95	0.42–2.14	0.97	0.86–1.26
F	3.61	1.09–11.97	1.48	1.01–3.72
G	2.26	2.09–2.44	1.22	0.93–2.33
H	1.57	0.50–4.89	2.06	1.95–2.35

disease. A method that can accurately measure a PVL of at least 4% is indispensable. However, the major problem in monitoring PVLs so far is difficulty in comparing the qPCR results across laboratories, because there is at most a 5-fold difference among laboratories in Japan (6). To standardize HTLV-1 qPCR, we considered that standardization could be achieved if all materials and methods were unified in all laboratories. However, unifying these methods might affect further development or improvement of HTLV-1 qPCR. In this study, using a panel of serial dilutions of TL-Om1 cells with PBMCs as the standard, we successfully matched the results of laboratories to the theoretical values by using correction factors determined by analysis of differences in the results of laboratories from the standard. Our standardization method minimized the differences in clinical PVL data between laboratories, which would greatly contribute to reliable measurements of PVLs and evaluation of PVLs.

Although our approach to standardization with a cell reference worked well in the majority of laboratories, some laboratories showed slight differences in the relative ratio between two independent years (Table 2). These laboratories also showed a relatively wide range of variations in the evaluation of periodic variations compared with the other laboratories (Fig. 3). Based on these results, one of the possible causes of the discrepancy between laboratories after the correction may be intralaboratory variability. A solution to improve the periodic reproducibility would be a run control that could monitor the trend of results over time.

To standardize HTLV-1 qPCR, it was important to prepare a suitable standard from a specific cell type. An example of a cell-based IS has been previously reported for quantitation of BCR-ABL mRNA to monitor the therapeutic response in patients with chronic myelogenous leukemia, in which the standard is prepared from K562 cells diluted in HL60 cells (20). Similar to the IS for quantitation of BCR-ABL mRNA, the formula of target samples for HTLV-1 qPCR and our TL-Om1 cell candidate standard are extremely similar. Thus, the TL-Om1 cell standard would behave similarly even though different measurement procedures are applied. Moreover, because the precise genomic properties of the HTLV-1 provirus and IC genes in TL-Om1 cells have been defined previously (10), the experiments in this study could be accomplished with absolute gene copy numbers.

There are several HTLV-1 molecular subtypes, including Cosmopolitan subtype A, Central African subtype B, Australo-Melanesian subtype C, and Central African/Pygmies subtype D. Rare cases found in Central Africa are classified as subtypes E, F, and G (2). Cosmopolitan subtype A is divided into four subsubtypes: Transcontinental (A), Japanese (B), West African (C), and North African (D). HTLV-1 subtypes in Japan include Cosmopolitan subtype A subsubtype Japanese (B) and subsubtype Transcontinental (A) (2). The sequences of HTLV-1 primers and probes used in Japanese laboratories are also completely homologous with the genomic sequence of HTLV-1 in TL-Om1 cells (NCBI accession number AB979451). The majority of laboratories use primers for the pX region of the HTLV-1 genome in their qPCR methods. Similarly, a comparison of primer sequences for HTLV-1 subtypes and TL-Om1 cells may be required at first. Otherwise laboratories can use primer and probe sets that have been specially developed to detect all HTLV-1 subtypes as well as HTLV-2 and HTLV-3 (21). However, we believe the standardization of HTLV-1 qPCR in a variety of areas would be achieved by using TL-Om1 cells as a standard material, because the sequence of the pX region is well conserved among HTLV-1 strains.

In this study, we analyzed four TL-Om1 cell dilutions. Relative ratios to the theoretical values were calculated by PLA. Although PLA is a major method to evaluate the potency of biological materials, the relative ratio calculated statistically by PLA and those simply calculated by data obtained at only one dilution of 0.8% TL-Om1 cells were extremely similar in all laboratories (Tables 2 and 4). The intralaboratory variations between testing periods in a laboratory were much larger than the differences in calculation methods (Fig. 3). This result shows that the standard is applicable to a wide range of concentrations for HTLV-1 qPCR. In the future, international or national standards for HTLV-1 qPCR could be possible using either a serial dilution or single dilution of TL-Om1 cells.

In conclusion, we confirmed that standardization of HTLV-1 qPCR can be achieved using a standard material prepared from TL-Om1 cells.

ACKNOWLEDGMENTS

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妊産婦診療におけるHTLV-1キャリア検出のための診断の進め方とキャリア妊婦支援の必要性

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はじめに

2010年から、妊婦健診時のHTLV-1抗体検査が公費で支援されることになりました。キャリア妊婦に対しては、経母乳母子感染予防の観点から人工栄養、凍結母乳栄養、3ヶ月以内の母乳栄養の3つを選択肢として呈示し、キャリア自らで栄養法を選択しています。この事業を30年間行うことにより、出生した児から将来発症する成人T細胞白血病（ATL）患者を日本から撲滅できます。この事業が開始して5年経過し、新たな変更点や問題点が生じてきましたので解説いたします。

問　どのようなことが判ったのでしょうか？

答　4つのことが判りましたので以下に順に述べます。

1）一次スクリーニング法での検査法の追加

一次スクリーニングでは、これまで粒子凝集（PA）法と化学発光酵素免疫測定（CLEIA）法が推奨されてきましたが、化学発光免疫測定（CLIA）法が、従来の検査法（PA、CLEIA）に匹敵する検査であることが判明したので、CLIA法も推奨できる検査法となりました（図1）。

2）一次抗体スクリーニングでは偽陽性が多い

日本産婦人科医会の調査により、69万人あまりの実態が明らかになり、一次検査陽性率は0.32%であること、このうち確認検査であるウエスタンブロット（WB）法が陽性であり、キャリアと同定できたのは50%であることが判明しました。またWB法陰性でキャリアでなかった症例は38.6%でした。すなわち一次抗体が陽性であっても、キャリアは半分しか含まれないということです（図1）。

3）WB法を行っても判定保留例が存在する

確認検査を行っても11.4%に判定保留となる症例が存在し、正確な判定と適切な授乳法の選択に苦慮する場合がまれでないことが判りました（図1）。臨床現場でどのように説明したら良いか判らないため、早急な解決法が求められます。

4）凍結母乳、3ヶ月までの短期母乳が実行困難であることが知られていない

厚生労働科学研究板橋班のデータでは、選択されている栄養法は人工乳33%、3ヶ月までの短期母乳57%、凍結母乳5%、その他5%でした。とくに短期母乳を選択した場合、途中で母乳を止められずに長期母乳となるケースがあること、凍結母乳では母乳がすぐに止まってしまい長期母乳できていないことなどの問題点が判りました。

図1　妊婦に対するHTLV-1スクリーニングの実態
（日本産婦人科医会のデータより）

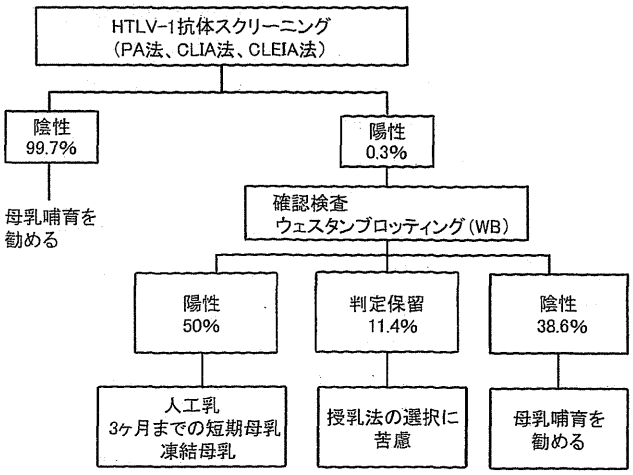
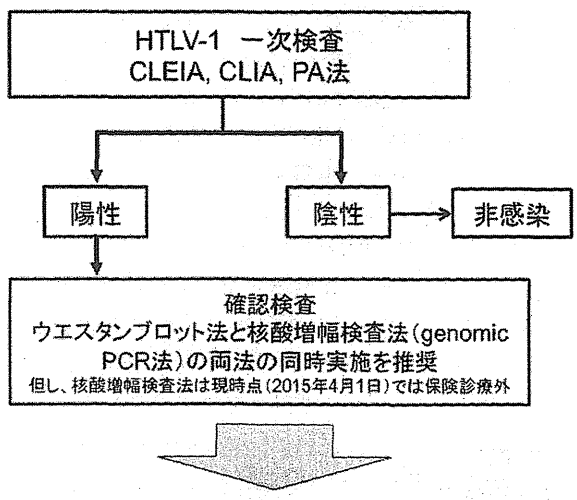


図2　診療におけるHTLV-1感染（症）診断のためのフローチャート（案）（参考論文3より引用）
HTLV-1感染（症）の診断法
（日本HTLV-1学会、日本産科婦人科学会、日本産婦人科医会、
日本周産期・新生児医学会　推奨検査手順）



☆　推奨法による判定確定法

PCR法 ＼ ウエスタンブロット法	陽性	判定保留	陰性
陽性	「陽性」と確定	「陽性」と確定	「陽性」と確定
陰性	「陽性」と確定	陰性もしくは検出感度以下	「陰性」と確定

問　確認検査をして判定保留であった場合、どのようにしたら良いのでしょうか？

答　一次検査で行われている抗体検査法は、非特異反応が起こることがあり、偽陽性となることがあります。そのためWB法を行い確認試験をしていますが、WB法の感度が低いため、判定保留となることがあります。判定保留者に対してHTLV-1核酸増幅検査法（PCR）法が正確な診断に有効であることが厚生労働科学研究板橋班と浜口班の共同研究により明らかになりました[1]。日本産婦人科医会の報告では判定保留者にPCR法を行ったところ21/60（35%）の陽性率でした。厚生労働科学研究板橋・浜口班ではPCR法での陽性率が26/135（19.3%）でした。両者を合わせると47/195（24.1%）のPCR法陽性率となります。PCR法陽性者に対する栄養法は人工乳、凍結母乳、3ヶ月までの短期母乳のいずれかを勧めて下さい。

またWB法判定保留でPCR法陽性者のHTLV-1プロウイルス量の中央値は0.01%（0.001～0.160%）と微量でした。末梢血中に5%以上のプロウイルス量があるとATLの発病のリスクが高くなりますので、WB法判定保留でPCR法陽性者の現時点でのATLやHAM（神経難病）のリスクは極めて低いといえます。

WB法判定保留かつPCR法陰性の場合は、非感染あるいは微少感染と判定します。微少感染とは、PCR法で検出感度以下（4コピー/100,000細胞以下）の感染を指します。微少感染では、長期母乳による育児を行っても人工乳栄養による育児での感染率とほぼ同等と考えられます。

これは、Biggarらの「母親の血中プロウイルス量が0.015%未満であると長期母乳しても母子感染率は3.4%である」という報告[2]と、日本での人工乳栄養による母子感染率が51/1,553（3.3%）ということを根拠としています。ただし、実際の感染率については、厚生労働科学研究（板橋班）が症例を集積中であり、結論は出ていません。上記の母子感染率を呈示し、長期母乳をしても人工乳栄養と母子感染率が変わらないことを説明し、不安を取り除いてあげ、キャリア本人の栄養法の選択を優先していただきたいと思います。

問　PCR法は現在保険収載されていませんが、どのようにしたら良いでしょうか？

答　厚生労働科学研究板橋班と浜口班ではWB法判定保留者に対して無償でPCR法を行い、その結果を依頼先に通知しています。ホームページ（http://htlv-1mc.org/）に各都道府県協力施設が掲載されていますので、可能であれば研究協力病院に紹介して下さい。研究協力病院が近くにない場合は、PCR法のメリットを説明し、希望される方には自費で検査（約¥20,000）を提出して下さい。

問　3ヶ月までの短期母乳や凍結母乳を選択した症例への支援はどうしたら良いでしょうか？

答　これまで産科医療施設は退院時に短期母乳や凍結母乳について簡単に説明するのみで、定期的なフォローアップを行ってきませんでした。また母乳を途中で止めることや搾乳を続けることの困難さが認識されていませんでした。3ヶ月で母乳を止める際には、3ヶ月に入った頃から徐々に人工乳に切り替えることが必要で、その間、乳房緊満や乳腺炎に悩むことも少なくありません。また4ヶ月に入った際、完全に人工乳に切り換っているか、乳房の状況はどうかなどを確認する必要があります。また、この間、積極的に褥婦を支援する必要があります。母乳外来を受診してもらったり、地域の保健師もしくは助産師に訪問看護してもらったり、地域でのサポートが、とても重要です。

凍結母乳を選択された際、搾乳がうまくいかないと、人工乳に頼ってしまい、すぐに母乳が止まってしまいます。凍結母乳のメリットは長期間投与できることですが、母乳管理が不十分であると乳汁分泌が早期に止まってしまい、せっかくのメリットが活かされません。母乳外来での管理や地域保健師、助産師の支援システム整備が必要です。

おわりに

各都道府県にはHTLV-1母子感染対策協議会が設置されていますので、各県でWB判定保留者に対しての対応や、出産後の乳房管理のシステムを決めていただき、地域でキャリア妊産褥婦を支援していただきたいと思います。またHTLV-1　PCR法検査が保険収載されれば、HIVスクリーニング法と同様に一次抗体検査陽性者にWB法とPCR法を同時に行うことにより、判定保留例がなくなり、適切な栄養管理法を速やかに呈示できるようになると考えられます[3]。すなわちWB法もしくはPCR法のいずれか1つが陽性であればキャリアと判断し、いずれも陰性であれば陰性（キャリアでない）と診断し、WB法判定保留でPCR法が陰性であれば陰性もしくは検出感度以下と判断します（図2）。PCR法の早期の保険収載が望まれます。

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HTLV-1 Carrier Mothers Need Continual Support to Accomplish Their Selected Nutrition Method for Mother-to-child Transmission Prevention in Kagoshima

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Abstract

INTRODUCTION: Since 2011, the nationwide mother-to-child transmission prevention program in Japan for HTLV-1 has recommended three nutritional methods: formula-feeding (FF), short-term breast-feeding (STBF) and frozen-thawed milk feeding. Here we clarify the support necessary for HTLV-1-positive mothers to accomplish their selected nutrition method in Kagoshima.

METHODS: We administered questionnaires to 93 HTLV-1-positive mothers to determine whether each baby was successfully fed by following the mother's selected nutrition method, and whether any problems were encountered. They were divided into 2 groups (FF and STBF) by chosen nutrition method and compared; the FF group comprised 23 women and the STBF group comprised 70 women.

RESULTS: We received responses from 70 of the 93 women enrolled. The success rate of accomplishing their selected nutrition method was lower in the STBF than the FF group, and the difficulty rate was higher in the STBF than the FF group. The major reasons for feeling a difficulty in accomplishing the STBF method were the lack of support for weaning, suffering from emotional stress, and inability to wean children from breast milk. In contrast, the major reason for feeling a difficulty in the FF group was not being understood by family members and/or neighbors.

CONCLUSIONS: HTLV-1-carrier mothers, especially mothers who selected the STBF method, needed continual support to accomplish their selected nutrition method for mother-to-child transmission prevention. It is necessary to improve the support environment for HTLV-1-carrier mothers in Japan without delay.

Key words: HTLV-1, mother-to-child transmission, breast-feeding

Introduction

The human T-lymphotropic virus type 1 (HTLV-1) is known to be the pathogenic agent of adult T-cell leukemia-lymphoma (ATL)[1,2)]. HTLV-1 is endemic in southern Japan, the Caribbean, Latin America and western Africa[3)]. Three main routes of HTLV-1 transmission are known. The first is mother-to-child transmission[4)], mainly due to ingestion of breast milk[5)]. The second is sexual transmission, mainly from men to women[6)]. The third is transfusion of blood that includes HTLV-1-positive cellular components[7)]. Of the three, breast-feeding is the predominant route of transmission.

Lymphocytes in breast milk are responsible for transmitting HTLV-1[5]. Refrain from breast-feeding is the best and easiest way to prevent mother-to-child transmission of HTLV-1. However, the advantages of breast-feeding over formula-feeding (FF) to prevent overall child morbidity and mortality have been well established[8], especially in developing countries[9]. There are a couple of approaches to prevent mother-to-child transmission of HTLV-1 via breast milk other than refraining from breast-feeding. One is freeze-thawing of breast milk; the infectivity of HTLV-1 in breast milk was lost during the freezing and thawing processes[10]. The next possible approach to reduce the milk-borne transmission is to limit the duration of breast-feeding[11-14]. This may be related to the protective effect of maternally derived anti–HTLV-1 IgG antibodies[15].

The prognosis for ATL is extremely poor, and no vaccine is yet available. Therefore, a public health system to prevent transmission from carrier mothers to infants is important. In 1990, the Japanese government decided not to introduce a nationwide system of HTLV-1 prevention based on the Health Labour Sciences Research Grant reports[16], which recommended implementation of appropriate prevention plans only in endemic areas. One of the reasons was that introduction of a prevention system in a non-endemic area might increase the risk of confusion. Another reason was that the number of HTLV-1 carriers was expected to decrease with or without intervention because there was a trend toward a reduced rate and duration of breast-feeding at that time.

However, the Health Labour Sciences Research Grant reports in 2010 suggested that the number of HTLV-1 carriers had not decreased, contrary to expectations, and that the distribution of HTLV-1 carriers had spread into non-endemic areas[17]. The phenomenon may be explained by the flow of the population from endemic rural areas to non-endemic major urban cities during the period of high economic growth in Japan. A nationwide system of HTLV-1 prevention is needed under such circumstances, and the Ministry of Health, Labour and Welfare introduced a nationwide mother-to-child transmission prevention program. It recommended three nutritional methods: FF, short-term breast-feeding (STBF) of <3 months after birth, and frozen-thawed milk feeding. Nation-wide screening of pregnant women for HTLV-1 infection was implemented in Japan in 2011[18].

On the other hand, in 1985, Kagoshima University began a study on the prevention of HTLV-1 transmission in collaboration with the local government of Kagoshima Prefecture. Kagoshima prefecture is located in Kyushu, in the south of Japan. Many HTLV-1-positive people live in Kyushu. In the 1985 study, Takahashi et al. determined that the seroconversion rate of STBF children was nearly equal to that of bottle-fed children[11]. Therefore, the prefecture-wide "ATL Prevention Decade Plan" to promote FF or STBF for HTLV-1 carrier mothers began in 1997. Approximately two-thirds of the HTLV-1 carrier pregnant women chose STBF, which was a higher rate than in other endemic prefectures[19]. In the previous report, in Kagoshima Prefecture, there were not many obstetrics facilities that cared for HTLV-1 carrier mothers when weaning from the breast[20]. Therefore, only 75% of HTLV-1 carrier mothers accomplished their selected nutrition method. In this study, we introduced a support system performed by visiting public health nurses. But it remains unclear whether HTLV-1-positive pregnant mothers are successfully implementing their selected nutrition method, especially STBF; in addition, whether any support is necessary for success is unknown.

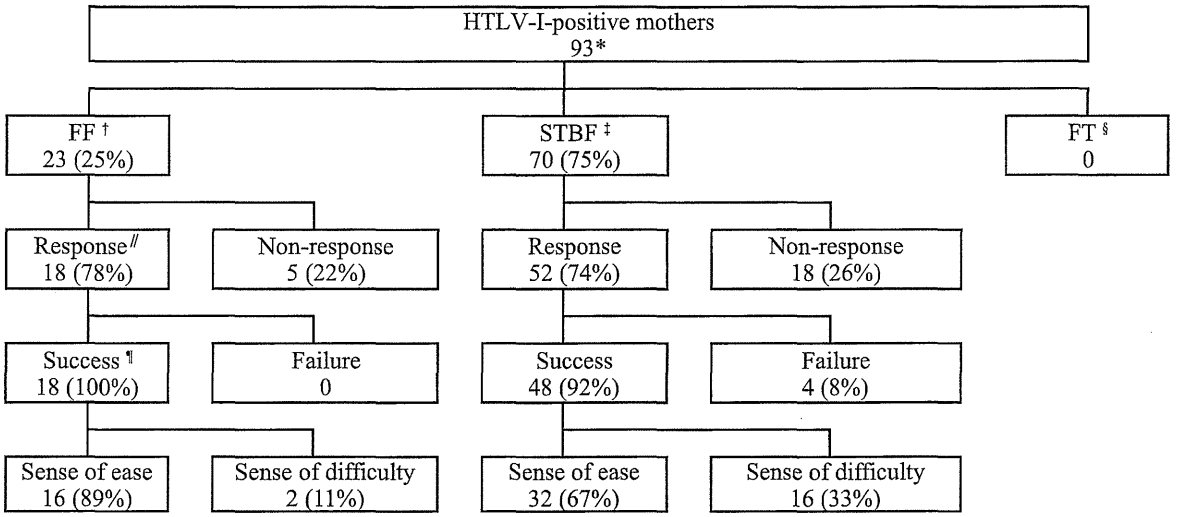
The aim of this study was to clarify the support necessary for HTLV-1-positive mothers to achieve success with their selected nutrition method in Kagoshima.

Methods

During the nation-wide screening of pregnant women for HTLV-1 infection, informed consents were obtained before screening for the antibody. Screening for HTLV-1 antibody was performed using the passive particle agglutination method (PA) or the chemiluminescent immunoassay (CLIA). Positive results were confirmed by Western blot analysis or an immunofluorescence assay[18]. The obstetricians or midwives in the obstetrics facilities in Kagoshima Prefecture recommended HTLV-1 positive pregnant woman to use one of three nutritional methods: bottle-feeding, STBF (<3 months), or frozen-thawed milk, as outlined in the HTLV-I mother-to-child transmission (MTCT) prevention health guidance manual compiled by the Ministry of Health, Labour and Welfare[18].

We obtained the cooperation of 48 of the 50 obstetrics facilities in Kagoshima Prefecture by the end of 2013. We visited these obstetrics facilities to obtain informed consent for this study from HTLV-1-positive pregnant woman between January 2013 and December 2013. We obtained informed consent from 93 HTLV-1-positive pregnant women. We investigated the choices made for nutrition methods before the delivery. No mother selected frozen-thawed milk (FT). Accordingly, the HTLV-1-positive pregnant women

Figure 1. Response rate, success rate, and rate of sense of difficulty in accomplishing the selected nutrition method"



* We visited obstetrics facilities in Kagoshima prefecture to obtain informed consent for this study from HTLV-1-positive pregnant woman between January 2013 and December 2013.
† FF: formula-feeding
‡ STBF: short-term breast-feeding (<3 months)
§ FT: frozen-thawed milk
// Response: We received replies from them.
¶ Success: They accomplished their selected nutrition method.

participants were divided into 2 groups: the FF group, consisting of 23 mothers; and the STBF group, consisting of 70 mothers.

In this study, support was introduced through visiting public health nurses after childbirth. They visited upon request from the mothers, with a frequency of approximately once per month. They provided consultation to help the mothers who were anxious about the nutritional status of their baby. We administered questionnaires to all 93 enrolled participants three months after delivery to determine whether each baby was successfully fed by following the selected nutrition method, and whether any problems were encountered. Frequency analysis was performed with the Fisher's exact test.

The study protocol was reviewed and approved by the Ethics Committee of Kagoshima University Graduate School of Medical and Dental Sciences (No.196).

Results

1. Response rate

We received replies from 52 of 70 (74%) STBF group subjects and 18 of 23 (78%) FF group subjects. Both groups had almost the same response rate (Fig.1). No significant differences were found between Kagoshima city and the urban area for the nutrition method choice.

2. Accomplishment rate

Successful use of the selected nutrition method was accomplished by 48 (92%) of the STBF group and the entire FF group. No significant difference was found between the two groups in accomplishment rate (Fig.1).

In addition, no significant differences were found in accomplishment rate between primiparas and multiparas.

3. Rate of feeling a difficulty in successful use of the selected nutrition method

In the STBF group, 16 (33%) found it difficult to achieve success with the selected nutrition method, as did 2 (11%) of the FF group. No significant difference in this rate was found

Table 1. The reasons for feeling a difficulty using the selected method

Group	Case number
Formula-feeding (FF) group	2
Feeling a difficulty	2
Lack of understanding from family members and/or neighbors *	2
Medical support was insufficient †	1
Short-term breast-feeding (STBF) group	20
Feeling a difficulty	16
Difficult to wean from the breast ‡	4
Trouble with breasts §	4
Sense of desolation after weaning from breast //	4
Medical support for how to wean was insufficient ¶	3
Lack of understanding from family members and/or neighbors	1
Failure to wean from the breast within 3 months	4
Difficult to wean from the breast	2
Medical support for how to wean was insufficient	1
Plan to go abroad **	1

(Multiple answers allowed)

* "Lack of understanding from family members and/or neighbors" included "It was difficult to explain to friends and relatives, excluding parents, why breast feeding was impossible" and "suffered from questions about breast-feeding."

† "Medical support was insufficient" means that the mother did not receive support as expected from a medical institution (The details were unwritten).

‡ "Difficult to wean from the breast" means STBF mothers found it considerably difficult to wean from the breast.

§ "Trouble with breasts" included mastitis and pain or discomfort caused by engorged breasts with weaning.

// "Sense of desolation after weaning from breast" included feelings of "I am not required by my baby" and "Why is it just me who cannot breast-feed?"

¶ "Medical support for how to wean was insufficient" means that the mother received insufficient support to wean from the breast.

** "Plan to go abroad" means that the mother expected to provide their baby passive immunity through the transfer of IgA antibodies found in breast milk in anticipation of traveling to a developing country.

between the two groups (Fig.1).

4. Reasons for feeling a difficulty in using the selected method

The reasons for feeling a difficulty for successful use of the selected nutrition method are listed in Table 1.

The major reason in the FF group was not being understood by family members and/or neighbors. In contrast, the major reasons for feeling a difficulty in accomplishing the STBF method were lack of support for weaning, suffering from emotional stress, and inability to wean children from breast milk. The major reasons for feeling a difficulty differed between groups.

Discussion

This study shows that HTLV-1-positive mothers found it considerably difficult to use their selected feeding method, regardless of the method chosen. Therefore, HTLV-1-positive mothers need much support to be successful with their selected method. Furthermore, the STBF group needed more support than the FF group, as evidenced by their lower accomplishment rate and the higher rate of feeling a difficulty with using the method despite the major reasons for feeling a difficulty differing between groups (Fig.1).

Because the total success rate (92%) of this study was higher than the rate (75%) of the previous report[20], the support by public health nurses might be effective. However, not all mothers could achieve their goals by using this support. Because the major reasons for feeling a difficulty with the STBF method were the lack of the support for weaning, suffering from emotional stress, and the inability to wean children from breast milk, further support is necessary (Table 1). Support by midwives may resolve these problems since they are specialists in childbirth, postpartum issues (including nursing), and women's health care. They can also help resolve the cognitive, emotional and technical problems of weaning. However, the economics of introducing such support is problematic.

On the other hand, the major reasons for feeling a difficulty

while using the selected method in the FF group was different that the STBF group in that feeling a lack of being understood by their family members and/or neighbors would make some mothers feel afraid of revealing their HTLV-1 carrier status (Table 1), which could occur if they attempted to explain why they refrained from breast feeding[19-21]. To eliminate prejudice against the HTLV-1 carriers, educational activities are required.

All 18 mothers in the FF group accomplished their selected nutrition method. According to our results, FF seemed to be the most reliable and easiest way to prevent mother-to-child transmission of HTLV-1. Additionally, in developed countries, including Japan, infectious diseases and malnutrition are not main causes of infant mortality. In light of this, the question of why the Japanese government recommends STBF as a nutrition method for these mothers is raised. Although the benefit of STBF is unclear, breast-feeding per se is beneficial to not only the health of the infants but also the health of mothers[8]. HTLV-1 causes ATL or HAM in only a minority (approximately 5%) of carriers after a long incubation period. In addition, the FF method cannot protect against all mother-to-child transmission. Approximately 3% of infants will be infected by their mothers, even if formula fed. The infection route of HTLV-1 when the FF method is used remains unknown[18]. Therefore, the optimal nutrition method for HTLV-1 carrier mothers is still controversial; more studies are needed to clarify this issue.

The limitations of this study include its small sample size and a possible selection bias. However, in 2012, the rate of pregnant carrier women was only 1.3% even in Kagoshima, which is located in a pandemic area. Theoretically, there are approximately 200 pregnant carrier women per year in Kagoshima. This study enrolled approximately half of all suspected carrier pregnant women in Kagoshima, which lends credence to the results.

In conclusion, HTLV-1-carrier mothers, especially mothers who chose the STBF method, need continual support to accomplish mother-to-child transmission prevention. Despite the fact that the Japanese government introduced a HTLV-1 mother-to-child transmission prevention health program by recommending three post-delivery nutrition methods, there exists no post-delivery system of support to achieve success in preventing transmission via those methods. In our study, 8% of STBF mothers failed in their selected method, as such, approximately 20% of those mothers' babies could become HTLV-1 carriers, of which 5% could suffer from associated diseases including ATL and HAM in future. However, if a support system were introduced, it would take time to see the effect. So even if there are unsolved problems, it is necessary to immediately improve the support environment for HTLV-1-carrier mothers in Japan. Any future trials in Japan will be important in informing optimal preventative strategies in other countries.

Disclosure of potenial conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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鹿児島県のHTLV-Ⅰ母子感染予防の栄養法達成のための母親への継続的な支援が必要

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【背景】2011年より栄養法として人工栄養、短期母乳、凍結母乳の3つが選択候補にあげられたHTLV-1母子感染対策は全国的な取り組みが開始された。鹿児島県においてHTLV-1陽性妊婦が出生前に決定した栄養法が実施完了できるのに必要な支援について明らかにする。
【方法】同意取得した出産3か月経過後の母親93名に質問票を送付し回収した。決定した栄養法で人工栄養、短期母乳の2群に分け、比較検討した。
【結果】93名のうち70名から回答を得た。選択した栄養法を完遂できた率は、人工栄養群にくらべ短期母乳群が低かった。また、栄養法を遂行するのに困難を感じた率は人工栄養群にくらべ短期母乳群が高かった。その困難を感じた主な原因は、短期母乳群では「断乳時の支援不足」、「感情的な苦しみ」、「子どもがお乳から離れない」であった。対照的に人工栄養群では「家族または周囲の人々の理解不足」であった。
【考案】HTLV-1母子感染対策のためにHTLV-1陽性の母親が出生前に選択した栄養法を完遂するには継続的な支援が必要である。それは短期母乳を選択した母親で特に認められた。
全国的な取り組みが開始された現在、HTLV-1陽性の母親を支援する環境を迅速に改善していく必要がある。