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Policy Forum

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The rural-to-urban migrant population in China: Gloomy prospects for tuberculosis control

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Summary **The migrant population is a population with a high risk of tuberculosis (TB) infection and transmission. Globally, migration is likely to have a significant impact on TB epidemiology, particularly in countries that receive substantial numbers of migrants from countries with a high infection burden. China, a country with the world's second highest TB burden, faces a considerable increase in the number of rural-to-urban migrants. This population has a significant impact on urban TB epidemics and is specifically targeted by national guidelines for TB control. TB control among the migrant population has had relatively poor outcomes. Barriers to detection and treatment have both financial and non-financial aspects, such as the "migratory" nature of the migrant population, their marginalized working and living environment, poor financial status, little awareness of TB, inadequate referral to TB dispensaries, and potential social stigma in the workplace. Currently, the free TB treatment policy has limited ability to relieve the financial burden on most migrant TB patients as would allow optimal outcomes of TB detection and treatment. Universal health insurance coverage and fostering of personnel in community-based primary health care for the rural-to-urban migrant population represent two pillars of successful TB control.**

Keywords: Migrant population, directly-observed treatment strategy (DOTS), tuberculosis control, China

1. Migrant population: High risk of TB epidemics

The migrant population is a population with a high risk of tuberculosis (TB) infection and transmission (*1-3*) and often faces barriers to access to appropriate health care for diagnosis and treatment (*4-6*). In low-incidence countries with large numbers of immigrants from countries with a high infection burden, migration is likely to have a significant impact on TB epidemiology as they receive substantial numbers of migrants from countries with a high burden of infection (*7*). In the United Kingdom, the increase in the rate of TB seems to be due to the fact that a high proportion of cases in the UK occur in the foreign-born, coupled with a large number of foreign nationals from countries with a very high incidence of TB (*8*). In the United States, the relative yield in finding and treating latent TB infection is particularly high among individuals from most countries of sub-Saharan Africa and Southeast Asia (*9*). In Spain, Garcia-Garcia *et al*. reported much worse treatment follow-up and outcomes among the immigrant population compared to the native population (*10*). Similar epidemiological characteristics are also reported in France, where the incidence rate among people born abroad was about seven times higher than that observed in people born in France; the incidence was also highest in districts with a high proportion of socioeconomically vulnerable individuals (*11*). Moreover, epidemics of multidrug-resistant tuberculosis (MDR-TB) hamper TB control, and the migrant population in particular has a high risk of developing multidrug-resistance (*3,12*).

In low-incidence countries, contact tracing in primary care, and particularly within ethnic communities, is reported to be much more effective and cost-effective at TB detection and control among

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the immigrant population than simple screening at entry (*13*). Truong *et al.* examined Tibetan immigrants undergoing medical screening in Minnesota in the US and they suggested that persons with a history of active TB require particularly close follow-up even in the face of negative Mycobacterium tuberculosis (*M. tuberculosis*) cultures (*14*). Specific strategies to manage TB within such a vulnerable population, including the improvement of social and work conditions, are crucial policy issues that must be considered in light of the increasing TB burden with immigration.

2. Epidemiological characteristics of TB in the Chinese migrant population

Second to India, China has one of the world's largest TB disease burdens. China has 1.3 million patients with TB annually, and TB cases in China account for approximately one quarter of cases worldwide (*15*). The Chinese government has a political commitment to TB control and had increased financing investment in recent years. As a result, from 2000 to 2009 the prevalence of TB declined from 466 cases per 100,000 population to 459 per 100,000, and active cases decreased from 169 per 100,000 to 66 per 100,000 (*16*). A strategy of a short course of directly observed treatment (directly observed treatment, short-course or DOTS) has been implemented in all counties nationwide since 2005. Active TB cases are cured by DOTS at a rate of over 90%, the global target for TB control (*17*). That said, major challenges lay ahead for the TB control program and related personnel, such as epidemics of MDR-TB and TB treatment and case management for the migrant population, given their propensity to change residences in the search for work.

In China, most migrants come from rural areas. The "hukou", or civil registration, system had kept people from moving from their permanent residences until the 1980s. After economic reforms, the hukou system no longer played an important role in dictating domestic residence and relatively large numbers of rural residents have moved to urban areas for more job opportunities. The size of the rural-to-urban migrant population has increased rapidly with economic development and urbanization. According to a national report released by the Chinese Academy of Social Sciences, the migrant population in China has reached 211 million (*18*). The national report indicated that the migrant population has an average monthly income of around 300 US dollars, and most migrants are engaged in high-risk industries and are not fully covered by social security and public services. Most migrant workers tend to be exposed to a higher risk of TB infection due to a poor living and working environment and poor nutritional status while their access to health care has often been seriously restricted by their registered residence, lack of knowledge about health care, and poor financial conditions (*19*).

Most of the migrant population in China comes from rural areas, where the number of TB patients accounts for approximately 80% of such patients nationwide and the prevalence of TB is almost twice that in urban areas (*15*). Since the 1990s when the TB registration system was extended to include migrants, the proportion of migrant TB patients has subsequently increased among TB patients nationwide (*20*). Similar to the epidemiological characteristics of TB in low-incidence countries with large numbers of immigrants, as mentioned earlier, the rural-to-urban migrant population has a higher TB incidence, a higher rate of newly registered cases of positive smears, and a higher rate of diagnosis and detection than permanent residents have (*20,21*). These trends have significantly affected TB epidemiology in urban China (*20,22,23*). In Beijing, the country's metropolitan capital, for example, a spatial analysis by Jia *et al*. suggested that TB tended to cluster in the migrant population and reemerged as the migrant population swarmed into Beijing; their analysis also indicated that the geographical differences in TB epidemics were mainly due to economic inequalities in districts (*22*).

3. Barriers to TB detection and treatment for the migrant population

Naturally, the migrant population's "migratory" nature hampers their ability to receive regular TB health care, which is particularly true for rural-to-urban migrants. A previous study by the current authors in rural Shandong Province, China suggested that rural-to-urban migrant workers tended to be one factor causing a low rate of DOTS success in rural areas and consequently led to a relatively high risk to spreading the disease (*24*). Moreover, the marginalized working and living environment of the migrant workers in urban areas further hampered their access to TB detection and treatment. In China, most rural-to-urban migrants undertake manual labor and personal services, which typically lack stability and security. These migrants are often paid little and are frequently laid off. Because they lack an urban residence, these migrants are not covered by the medical insurance scheme and are not entitled to the many other social benefits and services accorded to most urban residents. Although rural-to-urban migrants have increased health risks and are a vulnerable population in urban China, they tend to have less access to health care services than urban residents, resulting in unsatisfactory health outcomes (*25-27*).

Barriers to TB detection and treatment among the migrant population continue to have both financial and non-financial aspects. China recently implemented a free TB treatment policy that covered the migrant population as well. The free service package includes

diagnostic and treatment services, such as a free sputum smear test, a free X-ray examination upon one's initial visit, and free TB drugs in accordance with the standard protocol (6 months for new patients and 8 months for re-treated patients). That said, TB patients incur high medical costs when receiving prolonged treatment other than the standard protocol or undergoing repeated X-rays and blood tests, hospitalization, and receiving additional and sometimes unnecessary drugs to protect the liver and ancillary drugs (*28*). Revenue-driven practices in health facilities profoundly affect this free policy: given a financial incentive, health facilities often tend to keep patients longer than necessary or are reluctant to refer patients to TB dispensaries and they tend to prescribe unnecessary drugs or tests (*29*). Such practices can seriously increase the risk of MDR-TB. In a qualitative study in Shanghai, the current free TB treatment policy did not relieve the financial burden on migrant TB patients and cost was the major reason patients failed to complete treatment (*30*). Low-income patients are estimated to pay large amounts for medical treatment of TB, approximately equal to 42% to 119% of their annual household income (*31*). Without health insurance coverage, most migrant patients still pay huge amounts for TB diagnosis and treatment out-of-pocket.

In addition to economic status, several quantitative studies in China have reported that other barriers to access to TB care among migrant patients are lack of awareness and lack of knowledge in a marginalized population and inadequate and complicated referral to TB dispensaries (*23*,*32*). A large proportion of migrant TB patients tend to first visit a non-hospital facility for treatment of their symptoms, where doctors are more likely to delay due to a lack of skill and experience in identifying potential TB and where the facility has a financial incentive to keep patients at the facility (*33*). Although a quantitative approach has difficulty identifying these aspects, the qualitative study revealed that the migrant population enjoys weak protection of its rights in the workplace, and social stigma have an impact on the accessibility of TB care. According to Wei *et al*., many interviewees reported being laid off from work, being avoided by colleagues, and being fearful of revealing their TB status; the law was often too weak to protect the employment rights of migrants while they were ill (*30*).

4. Efforts needed to make a difference

Various issues in health policy need to be addressed to reduce the burden of TB among the rural-to-urban migrant population in China. For instance, in terms of detection, is screening effective and feasible in China? How can delays in diagnosis be avoided both by the patient and doctor? In terms of TB treatment and implementation of DOTS, how can the financial burden on migrant TB patients be reduced and how can better treatment outcomes be achieved with the current free treatment policy? How can the case management of migrant TB patients be improved? Evidence on the effectiveness of interventions is urgently needed.

TB screening has recently been implemented in certain populations and facilities in China. While low-incidence countries have large numbers of immigrants from countries with a high infection burden, countries with a high incidence have difficulty regularly screening the migrant population. The reasons for this are similar to various barriers to access to health care as mentioned earlier, including the migrant population's "migratory" nature, their marginalized working and living environment, poor financial status, little awareness of TB, inadequate referral to TB dispensaries, and potential social stigma in the workplace. That said, most of the migrant population comes from rural areas, where the prevalence and burden of TB is much higher. A tuberculin skin test during registration and active case identification could potentially enhance the case detection rate and reduce delays in diagnosis (*33*). Successfully eliminating these barriers, and especially the financial burden for the low-income population, will lead to significant changes in the risk of TB infection and burden of the disease on such a vulnerable population.

The financial burden represents one of the most significant constraints to the access of TB-related health care, both for detection and treatment. Although the current policy of free diagnosis and treatment has been implemented to remove the financial burden on TB patients, it seems to have a limited effect on improving the accessibility of detection and treatment, as the financial burden cannot be relieved due to the revenue-driven practices of health care facilities and out-of-pocket payment for a relatively large amount of drugs and tests not covered by insurance. Modification of the current package of free TB diagnosis and treatment services requires comprehensive evaluation of initial conditions, expected clinical and socioeconomic outcomes, and the cost-effectiveness of different options specifically targeting the migrant population in order to identify the types of additional health care that should be introduced, *e.g.* screening at registration, TB drugs or additional second-line drugs, and outpatient care *vs.* hospitalization. The question of whether the current package should be expanded should be based on an answer grounded in scientific evidence.

Several crucial policy issues with the health system and the social welfare system underlie the barriers to TB detection and treatment for the migrant population. Based on recent health policies in China, universal health insurance coverage and fostering of personnel in community-based health care for the rural-to-urban migrant population represent two pillars of successful TB control. Without universal health insurance coverage, the financial burden on migrant

patients cannot be relieved. Robust community-based health care, in terms of human resources, clinical proficiency, and sufficient financial investment to discourage revenue-driven practices, must be open to migrant patients. Without these two facets, TB cases in the migrant population cannot be promptly detected and properly managed. The Chinese government has a strong political commitment to fundamental strategies of TB control, but this effort also requires intensive study as well as determination of the effectiveness of different interventions to make a difference.

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Review

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Amino acid analysis of sub-picomolar amounts of proteins by precolumn fluorescence derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate

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Summary **Amino acid analysis (AAA) method is the most accurate methodology for absolute quantification of proteins. The conventional postcolumn method employing ninhydrin labeling of amino acids, which is adopted in automatic amino acid analyzer, is limited by low sensitivity. Therefore, a highly sensitive AAA method is required to confirm the data obtained from mass spectrometry or N-terminal sequence analysis. To increase the sensitivity of AAA, an analytical method based on precolumn derivatization with fluorescent 6-aminoquinolyl-carbamyl (AQC) reagent and separation of the AQC-amino acid derivatives by ion-pair chromatography using a reversed-phase column is reported herein. The sensitive analysis of low abundance proteins requires strict prevention of environmental contamination. In this review, we provide a protocol for high sensitivity amino acid analysis and show that the amino acid composition of bovine serum albumin below 100 ng, *i.e.*, 1.5 pmol, determined using the presented method, matched with the theoretical composition in with low standard deviations. These results suggest that the current AAA method is potentially applicable for highly sensitive analysis as a complement to mass spectrometry-based proteomics.**

Keywords: Highly sensitive amino acid analysis, protein quantification

1. Introduction

Amino acid analysis (AAA) is a classical analytical method that is essential for the absolute quantification of peptides and proteins. The quantitative accuracy of the method has rendered it useful for compositional analysis of proteins and biological materials, as well as for protein identification (*1*) and for confirmation of the data obtained from Edman degradation or mass spectrometry (*2*). Since the development of an automated amino acid analyzer by Moore and Stein in the mid-1950s (*3*), the range of application of AAA has been expanded to cover many fields such as protein science, pharmacology, physiology, and food chemistry. The currently employed amino acid analyzer remains unchanged in principle and operates based on a postcolumn derivatization method in which amino acids are separated on an ion-exchange column followed by derivatization with ninhydrin for detection of the amino acids.

The disadvantages of the postcolumn method include its low sensitivity, resulting from the need for visible absorbance detection of ninhydrin adducts, and the requirement for a long analysis time using ion-exchange chromatography. Currently, commercial auto-analyzers using ninhydrin detection require as much as several tens of micrograms of proteins (at least 2 nmol of each amino acid) and an analysis time of 2 h for compositional analysis. Fluorescent *o*-phthaldialdehyde (OPA) has been developed as an alternative postcolumn reagent, the use of which resulted in an order of magnitude increase in sensitivity (*4*). One major limitation of this methodology, however, has been that OPA reacts only with primary amines; consequently, the amino acids proline and hydroxyproline cannot be detected directly with OPA in the absence of an oxidizing agent such as hypochlorite under alkaline

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conditions (*5*).

As another derivatization alternative, the precolumn derivatization method in which amino acids are derivatized with chromophores or fluorophores before application to the column has been developed. Because the hydrophobicity of the amino acids is enhanced by the derivatization, the derivatized amino acids can be separated on a reversed-phase column, resulting in faster analysis and higher sensitivity than the conventional ion-exchange method. In 1984, a new precolumn method was reported, with a 1-pmol sensitivity, using phenylisothiocyanate (PITC). PITC reacts with amino acids to yield phenylthiocarbamyl (PTC) amino acids capable of UV absorbance (*6*). Furthermore, precolumn derivatization has enabled the sub-picomolar quantitation of amino acids with the development of fluorescent reagents such as OPA, 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F), 9-fluorenylmethyl-chloroformate (FMOC-Cl), and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) (See details in Section 2.2.). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection of pre-labeled amino acids has been also reported including pre-labeling by amine-reactive isobaric tagging reagents that include different mass group of stable isotope (*7*,*8*), by *p*-*N*,*N*,*N*-trimethylammonioanilyl-*N*'-hydroxysuccinimidyl carbamate iodide (*9*), and by AQC (*10*).

There are some approaches without any derivatization of amino acids for MS detection coupled to capillary electrophoresis (*11*) or LC using a pentafluorophenylpropyl-bonded silica column (*12*) or a porous graphitic carbon column (*13*).

Such highly sensitive AAA described above can complement MS-based proteomics of low abundance proteins. This review provides an overview of the methodology using AQC required for highly sensitive amino acid analysis of small quantities of samples while maintaining precise quantitative performance.

2. Quantitation of acid hydrolysates of protein

2.1. *Acid hydrolysis*

Proteins or peptides have to be completely hydrolyzed to yield free amino acids prior to AAA. Among the many potential methods of protein hydrolysis, such as the use of a strong acid as hydrochloric or methanesulfonic acid (*14*), a strong base such as sodium hydroxide (*15*), or enzymes (*16*), hydrolysis using hydrochloric acid (HCl) is currently universally applied because HCl can cleave peptide bonds completely and can easily be removed from hydrolysates by evaporation. Because constant boiling HCl retains the same content of HCl in the gas-phase and liquid-phase, protein samples can be hydrolyzed either by adding HCl directly to the samples (liquid-phase hydrolysis) or by distillation of HCl *in situ* and exposing the samples to gaseous HCl (gas-phase hydrolysis). In the case of highly sensitive analysis of small amounts of samples, gas-phase hydrolysis is preferred to prevent contamination from HCl.

Recently, we proposed an innovative hydrolysis method using cation exchange resin as solid acid catalyst, in which the sulfonate group of the resin hydrolyzes the peptide bonds (*17*). Hydrolysis of adsorbed proteins is achieved simply by heating the column packed with the resin after injection of proteins onto the column. Even though successful automation of the hydrolysis process was achieved using the packed catalyst, the manual protocol employing conventional hydrolysis by gas-phase HCl is explained in this review.

2.2. *Precolumn derivatization with fluorescent reagent*

As mentioned in the Introduction, AAA based on precolumn derivatization with fluorophores became a popular alternative to the postcolumn method. Sub-picomolar sensitivity has been realized by using fluorescent reagents and by carrying out reversed-phase chromatography that prevents dilution of peaks. NBD-F (*18*) and FMOC-Cl (*19*) are two examples of fluorescent derivatization reagents for amino acids. In general, the pre-column derivatization reagent is added in large excess. In the case where the reagent itself emits fluorescence, such as NBD-F and FMOC-Cl, the excess reagent has to be removed prior to chromatographic analysis because the fluorescence of the reagent interferes with detection of the amino acids. On the other hand, the use of OPA as a fluorescent derivatization reagent (*20*) precludes the need for reagent removal given that OPA only emits if it forms an adduct with an amino acid. OPA, however, has significant disadvantages in that it reacts only with primary amines in the absence of oxidants as described in the previous section, and it forms unstable adducts with amino acids. The instability of the OPA-amino acid adducts makes the use of OPA unsuitable for precolumn derivatization because the derivatized amino acids must be separated on a column after derivatization. For our purposes AQC was selected as a derivatization reagent (*17*). This reagent reacts with primary and secondary amines including proline and hydroxyproline to produce stable derivatives and excess reagent is immediately hydrolyzed to yield 6-aminoquinoline (AMQ) (*21*). Although AMQ has the same excitation maximum as the AQC-derivatives, the emission maximum of AMQ is different from that of AQC-derivatives. The difference in the emission maxima allows for the selective detection of the AQC-derivatives in the presence of a large excess of AMQ. Removal of the excess reagent prior to injection onto the reversed-phase column thus becomes unnecessary. We summarized features of these fluorescent reagents in Table 1.

Table 1. Comparison of fluorescent reagents for precolumn derivatization

Fluorescent reagent	Reactivity with secondary amines	Stability of amino acid adducts	Removement of excess reagent prior to chromatographic analysis
AQC	Yes	1 week at *r.t.*	Unnecessary
NBD-F	Yes	Unstable	Necessary
FMOC-Cl	Yes	30 h at *r.t.*	Necessary
OPA	No[a]	Unstable	Unnecessary

[a] OPA reacts with secondary amines in the presence of an oxidizing agent. Abbreviations: AQC, 6-aminoquinolyl-carbamyl; NBD-F, 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole; FMOC-Cl, 9-fluorenylmethyl-chloroformate; OPA, *o*-phthaldialdehyde.

2.3. *Chromatographic analysis*

In general, fluorescence intensity is stronger in organic solvents than in water due to fluorescence quenching by water molecules (*21*). Consequently, under the water-organic solvent gradient conditions employed in reversed-phase chromatography, the AQC-derivatives eluted at longer times, *i.e.*, the more hydrophobic amino acids, show higher intensity in a chromatogram. This difference in the intensity leads to a different response factor for each amino acid. Shindo *et al*. have developed an ion-pair chromatographic technique (*22*) that enhances their hydrophobicity of AQC-derivatives of hydrophilic amino acids and produces a similar response factor for all of the amino acids. This method is slightly modified in the current technique, as described in the Protocols section.

2.4. *Quantification of acid hydrolysates of protein*

A portion of the AQC-amino acids was separated on a reversed-phase column and the detected peaks in the chromatogram were calibrated with those of a standard solution of amino acids that contains 17 amino acids other than Trp, Asn, and Gln. Under the conditions of the conventional acidic hydrolysis, Asn and Gln are completely hydrolyzed to Asp and Glu, Trp is completely destroyed, Met is partially oxidized, Cys cannot be directly determined, and cystine is partially destroyed. We quantified Cys as half-cystine, Asp as the sum of Asp and Asn, and Glu as the sum of Glu and Gln. Usually, norvaline is added to a protein solution prior to hydrolysis as an internal standard for corrections of physical or chemical losses and variations of amino acids during hydrolysis. For labile amino acids, Ser and Thr, or for slow cleavage amino acids, Ile and Val, time-course hydrolysis is often employed. By extrapolating the observed amounts of Ser and Thr to zero-hour hydrolysis time, the starting amounts of these amino acids can be determined. In the case of Ile and Val, the observed amounts will reach a plateau as the hydrolysis time advances and the value at the plateau is regarded as the true amount of these amino acids (*23,24*).

There are two methods for calculating the amount of protein from the quantified amino acids. In one method, the protein amount is computed by adding all the values of multiplication of the observed amount of each amino acid by its molecular weight. Although the amount of protein can be determined without information on the amino acid sequence of the proteins, amino acids destroyed during hydrolysis cannot be considered. In the second method, the protein amount is calculated by dividing the observed amount of each amino acid by its theoretical residue number calculated from the amino acid sequence of the protein. Under ideal conditions, the obtained protein amount is the same when calculated from each amino acid. Because some amino acids are, however, partially or completely destroyed as described above, and incomplete bond cleavage between Ile-Val, Val-Ile, Val-Val, and Ile-Ile are known (*25,26*), well-recovered amino acids are chosen to quantify the amount of protein. The protein amount was determined herein by averaging the protein contents calculated from the recovery of Ala, Phe, and Leu.

2.5. *Compositional analysis of bovine serum albumin*

Bovine serum albumin (BSA, 500 ng) was hydrolyzed, and two portions (45 ng and 100 ng) of the BSA hydrolysates were analyzed. The amino acid compositions of the respective hydrolysates are listed in Table 2. In both experiments, three samples were independently analyzed, and the standard deviation of each mean was calculated as $(\Sigma(\text{experimental value} - \text{average value})^2/2))^{1/2}$. The sequence of BSA reported in the Swiss-Prot database with accession number P02769 [25-607] was used to obtain the theoretical BSA composition, which was calculated by considering the conversion from Asn/Gln to Asp/Glu and the complete destruction of Trp.

As listed in Table 2, the obtained amino acid composition was in agreement with the theoretical composition, except that cystine was partially destroyed. In our laboratory, the setting of samples in the reaction vessel is carried out in a N_2-saturated hood and the reaction vessel is degassed sufficiently to prevent the oxidation of amino acids. As listed in Table 2, Met was quantitatively recovered in our system.

2.6. *Response linearity*

It is important to demonstrate response linearity in the rage of analytical interest for quantification. There are

two factors that affect the linearity: (*i*) the linearity of the photomultiplier tube (PMT) response of the fluorescence detector and (*ii*) the reaction linearity of the AQC reagent.

The PMT response depends on the electric voltage that is applied to the PMT. To evaluate the linearity of the PMT response, various amounts of Arg, in the range from 10 fmol to 5 nmol, were derivatized. The area under the Arg peaks was plotted against the injected amount as shown in Figure 1A. Under the experimental conditions used in the reviewed method, a linear response of the PMT was obtained in the Arg concentration range of 100 fmol to 500 pmol. Next, various amounts of amino acids standards were derivatized with the same amount of AQC, in the range from 50 pmol to 1 nmol, corresponding to total amino acid contents of 850 pmol to 17 nmol. A portion of the derivatized standard was injected in order not to exceed the linearity range of PMT. As shown in Figure 1B, linearity was maintained up to a concentration of 14 nmol of amino acids; at increasing concentrations of amino acids, the amount of AQC reagent was insufficient for complete derivatization and linearity was lost.

Table 2. Amino acid compositions after gas-phase acid hydrolysis of BSA at 110°C for 20 h

Amino acid[a]	Theoretical composition (%)	Estimated composition (%)[b]	
		45 ng of BSA	100 ng of BSA
Arg (R)	4.0	4.0 ± 0.03	4.1 ± 0.04
His (H)	2.8	3.0 ± 0.01	2.9 ± 0.00
Ser (S)	4.8	5.0 ± 0.07	4.8 ± 0.05
Gly (G)	2.8	3.0 ± 0.01	3.0 ± 0.05
Thr (T)	5.7	5.8 ± 0.08	5.9 ± 0.04
Pro (P)	4.8	5.0 ± 0.17	5.0 ± 0.12
Ala (A)	8.1	8.1 ± 0.02	8.2 ± 0.04
Asp (D)	9.5	9.5 ± 0.11	9.2 ± 0.05
Glu (E)	14	15 ± 0.10	15 ± 0.04
Tyr (Y)	3.4	3.3 ± 0.04	3.3 ± 0.02
Val (V)	6.2	6.1 ± 0.04	6.1 ± 0.01
Met (M)	0.69	0.74 ± 0.01	0.76 ± 0.02
Lys (K)	10	10 ± 0.10	10 ± 0.04
Ile (I)	2.4	2.3 ± 0.02	2.4 ± 0.02
Leu (L)	10	11 ± 0.13	11 ± 0.04
Cys (C)[c]	6.0	3.7 ± 0.09	3.6 ± 0.01
Phe (F)	4.6	4.7 ± 0.03	4.7 ± 0.02

[a] Letters in parenthesis are one-letter codes of amino acids. [b] Values listed are the means and standard deviations of three independent experiments (See text for experimental conditions). [c] Cysteine was quantified as half-cystine.

3. Materials

For the analysis of small amounts of samples, a clean hood and polymer gloves are needed in order to prevent contamination during sample preparation.

3.1. *Reagents and apparatuses in a clean hood*

1) 6 mm × 32 mm borosilicate glass tubes (Crimp Top Vials, P/N: 03-CVG, Chromacol, UK) for samples and 27.75 mm × 70 mm borosilicate glass vials (P/N: 224832, Wheaton, NJ USA) as hydrolysis vessels (*See Note 1*).
2) Mininert valves (No.SC-24, P/N: 10130, Pierce, IL USA) for gas phase hydrolysis.
3) Centrifugal concentrator (Micro Vac MV-100, TOMY Seiko Co., Ltd., Tokyo, Japan) (*See Note 2*).
4) Heat block bath (Thermo Alumi bath ALB-121, Scinics Co., Tokyo, Japan) with an aluminum block possessing 28 mm diameter and 100 mm depth holes and an aluminum block cover.
5) Constant boiling HCl prepared from purchased HCl (P/N: 086-03925, Wako Pure Chemical Industries, Ltd., Osaka, Japan).
6) Crystalline phenol (P/N: 162-17361, Wako) to prevent halogenation of Tyr (*27*).
7) Clean forceps and a spatula, pipettes and disposable tips.
8) Vortex mixer (N-20M, Nissin Scientific Co.,

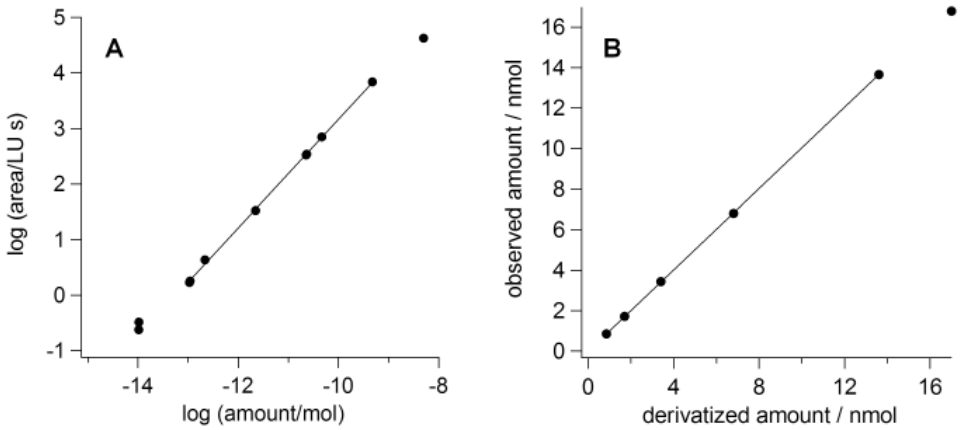

Figure 1. Linearity of the analysis: Detector response and AQC reagent reactivity. (A) Linearity of photomultiplier response. Peak areas of various amounts of arginine were plotted against injected amount. **(B)** Linearity of reactivity of AQC reagent. Observed total amino acids of standard solutions were plotted against those of standard solution derivatized with same amount of AQC reagent.

Tokyo, Japan).

9) Pre-cut polyethylene snap cap, 8 mm, for the Chromacol glass tube (P/N: 8-PEC1X, Chromacol).
10) Derivatization reagent (AQC powder dissolved in acetonitrile to provide a 3 mg/mL solution ca. 10 mM): AQC was included in a commercial kit (AccQ Fluor Reagent Kit, P/N: WAT052880, Waters Co., MA, USA) or was synthesized according to the literature (*14*). Briefly, 6-aminoquinoline (1.5 g, 10 mmol, P/N: 275581, Sigma-Aldrich Co., MO, USA) dissolved in 50 mL of dry acetonitrile (P/N: 013-15545, Wako) was added dropwise to a refluxing solution of di(*N*-succinimidyl)carbonate (3 g, 12 mmol, P/N: 43720, Sigma-Aldrich) in 100 mL of dry acetonitrile. The resulting crystals were filtered, washed, and recrystallized from dry acetonitrile and the synthesized AQC was stored in a dry place (*10*).
11) 0.2 M borate buffer (pH 8.8) made by weighing 0.76 g of sodium tetraborate (P/N: 71999-250G, Sigma-Aldrich) in a pyrolyzed glass vial and adding 9 mL of MilliQ water with heating for dissolution. The solution was then titrated to pH 8.8 with 6 N HCl, and MilliQ water is added to increase the weight of the solution to 10 g.
12) 10 pmol/μL amino acid standard solution, diluted from the commercial mixed standard stock solution (amino acid standard H containing 17 amino acids (2.5 mmol/L each except for 1.25 mmol/L of cystine) other than Trp, Asn, and Gln, P/N: 20088, Pierce).
13) 20 mM HCl: Mix 3 μL of constant boiling HCl with 997 μL of MilliQ water.

Note 1. Glassware was set in a deep, glass Petri dish and heated to 550°C for 3 h in a Muffle furnace to pyrolyze organic substances.
Note 2. The concentrator is custom-made for acid resistance.

3.2. *Chromatographic analysis*

1) Acetonitrile (HPLC grade, P/N: 01031-2B, Kanto Chemical Co., Inc., Tokyo, Japan).
2) Tetrabutylammonium bromide (TBA-Br, P/N: 207-04335, Wako) as ion-pair reagent.
3) Phosphate for chromatography: sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$, P/N: 106346) and disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$, P/N: 106580) purchased from Merck KGaA, Darmstadt, Germany.
4) Elution buffer A: 95% of 30 mM phosphate buffer (pH 7.3) containing 5 mM TBA-Br and 5% acetonitrile (HPLC grade), elution buffer B: 50% of 30 mM phosphate buffer (pH 7.3) and 50% acetonitrile (HPLC grade).
5) Column: InertSustain C18HP, 3 μm, 3.0 mm × 250 mm, (P/N: 5020-14426, GL Sciences Inc., Tokyo Japan) with pre-column filter A-701 (Upchurch Scientific, WA, USA).
6) Agilent 1200 series HPLC system equipped with degassers (G1379B), binary pump (G1312B), an autosampler (well-plate sampler, G1367A or standard sampler, G1329B), a column oven (G1316B), a diode-array detector (G1315C) with flow cell of 1.7-μL volume, 6-mm cell path length, and a fluorescence detector (excitation at 250 nm and emission at 395 nm, G1312A) with flow cell of 8-μL volume. Control of the HPLC and data treatment was done using ChemStation (Agilent Technologies, Inc., Santa Clara, CA, USA) software.

3.3. *Other reagents and apparatuses*

1) Bovine serum albumin (BSA, A7638) was purchased from Sigma-Aldrich. BSA solution was prepared by dissolving BSA in MilliQ water.
2) Heat bath for derivatization at 55°C with an aluminum block possessing 6 mm diameter holes (DRI-BLOCK DB-1L, M & S Instruments Inc., Osaka Japan).
3) Electronic balance (ER-182A, minimum weight = 0.01 mg and FX-3000, minimum weight = 0.01 g, A & D Co., Ltd., Tokyo, Japan).
4) Static eliminator (AD1683, A & D) for weighing samples.
5) Ultrasonic cleaner (AU-80C, Aiwa Medical Industry Co., Ltd., Tokyo, Japan) for dissolving AQC in acetonitrile.
6) Rotary vacuum pump (GCD-051X, ULVAC Inc., Kanagawa, Japan) for centrifugal concentrator with trap in Dewar vessel including liquid nitrogen.

4. Protocols

4.1. *Sample preparation*

In order to obtain accurate compositions using AAA, protein samples must be purified. It is also recommended that substances that may interfere with derivatization, such as salts or detergents, be removed if possible in the case of the precolumn method.

1) Weigh the purified proteins by the combined use of the microelectronic balance and the static eliminator.
2) Dissolve the protein in MilliQ water to provide a sample solution with a typical concentration in the range of 0.2-1 μg/μL.

4.2. *Hydrolysis*

1) Use a pipette to place the protein sample solution (typically less than 2 μg) or amino acid standard solution (typically containing 50 pmol of each amino acid) into a clean 6 mm × 32 mm glass tube

containing 50 pmol of norvaline as an internal standard. Prepare a clean glass tube containing 50 pmol of norvaline only as a control blank of the hydrolysis.

2) Evaporate the protein solution, the standard solution, or blank control to dryness using the centrifugal concentrator with the rotary vacuum pump.
3) Place the sample tube into the glass vial containing 200 μL of constant-boiling HCl and a piece of phenol crystal (ca. 1-2 mg).
4) Seal the vial after evacuation for a few minutes, by using the Mininert valve.
5) Hydrolyze the sample at 110°C for 20 h in the heat bath.
6) Remove the vial from the heat bath and allow it to cool. Open the vial and remove the tubes with the forceps. Wipe the outside of the tubes with a Kimwipe. Remove excess HCl using the centrifugal concentrator with the rotary vacuum pump to prevent interference with the subsequent derivatization.

Note: The procedural steps from 3 to 5 are preferably performed in a glove box, in an inert nitrogen atmosphere, given that atmospheric oxidation of amino acids results in poor quantitation.

Table 3. Elution program for separation of AQC-amino acids

Time (min)	Concentration of buffer B (%)
0	2
3	7.3
44	60
45	99
47	99
48	2
62	2

See text for details of buffer composition and flow rate.

4.3. *Derivatization*

4.3.1. *Calibration standard*

1) Place 5 μL of 10 pmol/μL standard, norvaline, and carboxymethylcysteine solutions into a clean 6 mm × 32 mm glass tube; evaporate to dryness and add 10 μL of 20 mM HCl.
2) Add 30 μL of 0.2 M borate buffer and mix.
3) Add 10 μL of a 3 mg/mL AQC solution, vortex immediately after the addition.
4) Seal the tube using aluminum foil and the pre-cut polyethylene snap cap.
5) Heat the vial in the block heater at 55°C for 10 min.

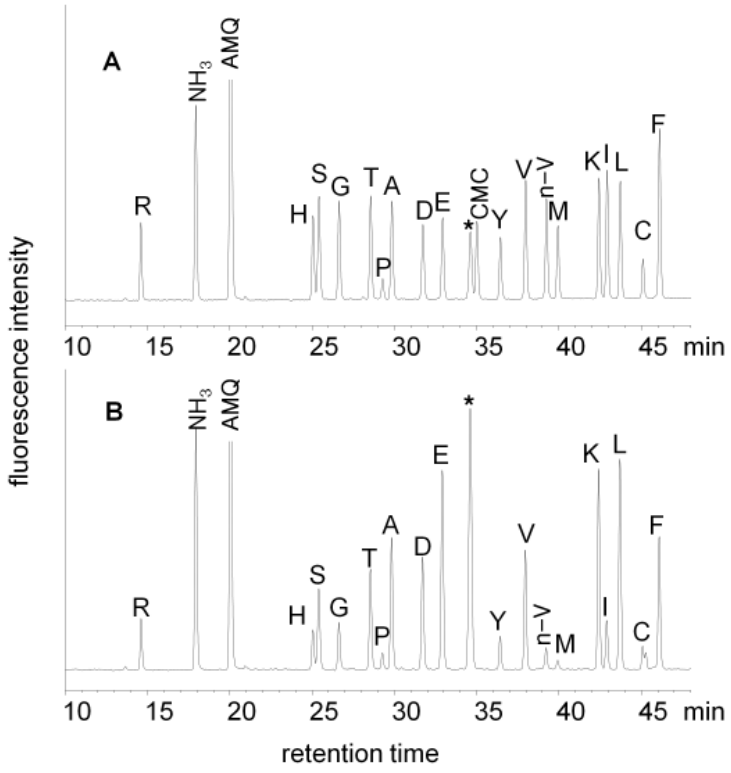

Figure 2. Chromatogram of AQC-amino acids of standard and BSA hydrolysate. (A) Chromatogram of 5 pmol each of the AQC-amino acid standard. **(B)** Chromatogram of 150 fmol of hydrolyzed BSA. The expansions of the one-letter abbreviations of the AQC-amino acids are indicated in Table 1. Peak labeled C is cystine. Abbreviations: NH_3, ammonium; AMQ, 6-aminoquinoline; CMC, *S*-carboxymethylcysteine; n-V, norvaline. Peak with an asterisk is unknown chemical peak.

4.3.2. *Control blank for derivatization*

1) Place 5 μL of 10 pmol/μL norvaline in a clean glass tube. Add 5 μL of 20 mM HCl.
2) Add 30 μL of 0.2 M borate buffer and mix.
3) Add 10 μL of AQC solution and vortex.
4) Seal the tube using aluminum foil and the pre-cut polyethylene snap cap.
5) Heat the vial in the block heater for 10 min at 55°C.

4.3.3. *Sample hydrolysis*

1) Add 10 μL of 20 mM HCl to the sample tube and vortex.
2) Add 30 μL of borate buffer and vortex.
3) Add 10 μL of AQC solution and vortex.
4) Seal the tube with aluminum foil and the pre-cut polyethylene snap cap.
5) Heat the vial in the block heater for 10 min at 55°C.

4.4. *Chromatographic analysis*

1) Analyze AQC-amino acids using the elution program shown in Table 3. Set flow rate at 0.4 mL/min and column temperature to 42°C (*See Note*). Entire separation is completed in 47 min as shown in Figure 2. Including column re-equilibration, the total run time for a typical analysis is 62 min.
2) Injection volume of sample solution, standard or blank control solution: typically 5 μL.

   Note: Using the 3.0 mm × 250 mm (3 μm)-column under these conditions, the system pressure is about 190 bar.

4.5. *Quantitation of amino acids*

1) Evaluate the amounts of 17 amino acids in the protein sample by calculating the ratio of the peak-height of an amino acid in the sample to that of the same amino acid in the standard.
2) Calculate the percentage composition of the amino acids by dividing the amount of each residue by the total amount of amino acids and multiplying by 100.

5. Discussion

Highly sensitive AAA of low abundance proteins can be achieved by using fluorescent reagent, AQC, for derivatization of amino acids. For the high sensitivity AAA, prevention of environmental contamination is critical to obtain a precise result. Especially, sample-handling procedures before proteins are hydrolyzed to their component amino acids require extra attention because the amino acids of contaminant proteins cannot be distinguished from those of the sample protein. In this review, we provided the protocol for the high sensitivity AAA. Using the protocol, the amino acid composition of BSA less than 100 ng, *i.e.*, 1.5 pmol, was determined and the composition was matched the theoretical composition with low standard deviations.

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Review

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UVA-induced protection of skin through the induction of heme oxygenase-1

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Summary **UVA (320-400 nm) and UVB (290-320 nm) are the major components of solar UV irradiation, which is associated with various pathological conditions. UVB causes direct damage to DNA of epidermal cells and is mainly responsible for erythema, immunosuppression, photoaging, and skin cancer. UVA has oxidizing properties that can cause damage or enhance UVB damaging effects on skin. On the other hand, UVA can also lead to high levels of heme oxygenase-1 (HO-1) expression of cells that can provide an antioxidant effect on skin as well as anti-inflammatory properties in mammals and rodents. Therefore, this review focuses on the potential protection of UVA wavebands for the skin immune response, instead of mechanisms that underlie UVA-induced damage. Also, the role of HO-1 in UVA-mediated protection against UVB-induced immunosuppression in skin will be summarized. Thus, this review facilitates further understanding of potential beneficial mechanisms of UVA irradiation, and using the longer UVA (UVA1, 340-400 nm) in combination with HO-1 for phototherapy and skin protection against sunlight exposure.**

Keywords: UVA, heme oxygenase-1, immunoprotection

1. Introduction

The solar ultraviolet (UV) is a spectrum of sunlight that causes photoaging, DNA damage, gene mutation, and even skin cancer (*1*). UV light that reaches the earth is divided into ultraviolet A (UVA, 320-400 nm) and ultraviolet B (UVB, 290-320 nm), and the UVA waveband can be further divided into UVA1 (340-400 nm) and UVA2 (320-340 nm) (*2,3*). UVB constitutes less than 10% of solar UV, and it is generally thought to be the most deleterious radiation of sunlight and the major wavelength involved in erythema, immunosuppression and carcinogenesis outcomes (*4*). UVA constitutes over 90% of solar UV. It can penetrate deep into skin, generate reactive oxygen species (ROS) through reacting with cellular chromophores, initiate photochemical and photobiological events (such as oxidation of various molecules and DNA damage), and consequently cause immunosuppression (*1,2,5*). It was found that UVA could cause immunosuppression in human skin, and sunscreen studies also indicated that a broad-spectrum sunscreen (blocking both UVA and UVB) provides better protection compared to a narrow blocker (only UVB), thus showing an important role of UVA in inducing immunosuppression (*6-8*). However, recently, a growing number of studies indicated that UVA radiation could provide immunoprotection and also inhibit UVB-induced immunosuppression through modulation of various cutaneous cytokines and enzymes, such as heme oxygenase-1 (HO-1) (*2,9,10*).

HO-1 is a potent anti-inflammatory intracellular mediator, which catalyzes the degradation of heme to iron, biliverdin, and carbon monoxide (CO) (*11*). High expression of HO-1 is observed in many types of cells after stimulation by UVA radiation, nitric oxide, heavy metals, and many other oxidants (*1,2*). Induction of HO-1 causes anti-inflammatory and anti-apoptotic

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properties. Especially, the anti-inflammatory capacity of HO-1 can exert immunomodulation of cell responses and alleviate a number of chronic inflammatory diseases (*12*). Tyrrell and Reeve suggested that the protection of skin by UVA irradiation with appropriate doses and wavelengths may be linked to UVA-induced HO-1 (*2,13*). In recent years, studies of the mechanism of UVA induced HO-1 immunoprotection have made great progress, for example, IL-6 and estrogen receptor-β (Er-β) are involved in mediating immunoprotection by UVA radiation, and gender difference is one of the factors that influence UVA-induced immunoprotection (*9,14,15*). So it is necessary to summarize recent developments of studies of UVA induced HO-1 expression and its protective effects on skin. In this article, HO-1 immunoprotection in skin, and its relationship with UVA induced immunoprotection will be elaborated, and UVA phototherapy will also be briefly mentioned due to its beneficial role in treating some skin diseases.

2. The relationship between UVA waveband, dose and immune function

There is a great deal of disagreement on UVA-induced immune function, which could be attributed to UVA waveband and dose. The wide spectral waveband of UVA (320-400 nm) could react with different chromophores in skin, leading to different effects on the immune system of skin (*16*). It was found that pretreatment with UVA1 (340-400 nm) may offer partial protection against UVB-induced decrease of immunization reaction to epidermis allergens (*17*). In another study, the result indicated that UVA1 did not elicit immunosuppression, however, UVA did induce immunosuppression that might be caused by UVA2 (shorter wavelength of UVA, 320-340 nm) (*18*). UVA1 and UVA2 might show differences in the regulation of immune functions: UVA1 may provide protection in the immune system; UVA2 is related to UVB in some parts of the immune response, such as immunosuppression, since their wavebands are adjacent (*2,18,19*).

In addition to the wavelength, UVA irradiation with different dosages may cause various biological effects on the immune system. Halliday *et al.* found that irradiation with a low dose of UVA (8.4 kJ/m^2) on humans could enhance immune memory; a medium dose of UVA (16.8 kJ/m^2) might have immunosuppressive activity, but higher doses of UVA (*i.e.*, 33.6 kJ/m^2) could protect skin from UVB damage (*20*). Similar results have been obtained using mice to show that UVA acts as an immunosuppressor at a lower dose (18 kJ/m^2) and an immunoprotector at a higher dose (34 kJ/m^2) (*21*). An *in vivo* study, using hairless mice, identified UVA doses, between 16 and 580 kJ/m^2, could provide immunoprotection against UVB-induced immunosuppression. However, UVA might be immunosuppressive at doses over 600 kJ/m^2 (*22*). We suggest that the discrepancy of immuno-functions among those studies may be due to the non-uniformity of the UV radiometer used for dose measurement and the definition of dose levels (low, medium and high). The different mice strain may also account for the discrepancy as well.

Recently, our study indicated that the increase of HO-1 levels by interference of Bach1 (BTB and CNC homology 1) might protect human skin keratinocytes from damage by a high dose of UVA (400-500 kJ/m^2) irradiation, while this increment had no effect on the protection for low to medium doses (100 and 250 kJ/m^2) (*23,24*). Nevertheless, *in vivo* studies of UVA immune responses mostly adopted mice models, and the immune responses of human skin are currently unclear due to too few studies.

3. UVA induced HO-1 protection in skin cells

UVA radiation alters a series of antioxidant pathways in skin cells, and one of the robust pathways is through the induction of HO-1 expression. HO-1 plays a vital role in protecting various cells and tissues against oxidative stress by virtue of its anti-inflammatory, anti-apoptotic, and anti-proliferative features (*2*). Also, it is a powerful immunomodulator which could eliminate some inflammatory responses (*25*). HO-1 can be induced not only by UVA radiation but also by many other stimuli, including heavy metals, endotoxin, H_2O_2, heat shock, LPS, inflammatory cytokines, and other antioxidants (*1,2,26*); The level of HO-1 expression depends on the type of cells. In this section, we will introduce the functions of HO-1 in skin and its role in UVA-induced immunoprotection.

3.1. *HO-1 and its functions in skin*

HO-1 is a rate-limiting enzyme in heme metabolism and can cleave heme into three products: biliverdin, CO, and ferrous iron. Biliverdin is further reduced by biliverdin reductase to bilirubin. Biliverdin and bilirubin are recognized as potent antioxidants by scavenging ROS (*27*). CO, as biliverdin, contributes substantially to the anti-inflammatory properties of HO-1 by suppressing pro-inflammatory cytokines and also has tolerogenic actions in adaptive immune responses (*11,13*). Ferrous iron is released during the breakdown of free heme by HO-1, but this molecule is rapidly removed by ferritin. It is suggested that increased ferritin expression in conjunction with HO-1 expression may contribute to the additional protection by HO-1 (*28-30*).

The activation of HO-1 has been recognized as a sensitive marker for oxidative stress to cells, and it is involved in an adaptive protective response against oxidative damage, such as UV irradiation (*1,2*). Recently, many studies showed that HO-1 might

provide protection through its upstream activation genes, such as transcription enhancer Nuclear factor-erythroid-2-related factor 2 (Nrf2). For example, Hirota found that Nrf2 might play an important role in the protection of the skin against UVA irradiation in mice fibroblasts (*31*). Later, Nrf2-driven HO-1 expression was found to protect mouse skin cells from oxidative carcinogenesis (*32*). Our studies also showed that Nrf2 might protect human skin fibroblasts and keratinocytes from UVA irradiation (*23,33*). These studies indicated that Nrf2-driven HO-1 expression may play a role in cellular protection. So far, these studies focused on the upstream regulation of HO-1. However, the protection provided by HO-1 through its downstream genes is yet to be identified.

3.2. *HO-1 in UVA-induced immunoprotection*

The induction of HO-1 may play an important role in the limiting inflammation and immune activation. For example, cutaneous HO-1 expression might represent a potent therapeutic approach for the treatment of T cell-dependent inflammatory dermatoses (*34*). It is also shown that HO-1 has immunomodulatory capacity in adaptive responses, and deficiency of HO-1 leads to development of chronic inflammatory pathology and widespread oxidative tissue injury (*35*). Lately, a study indicated that HO-1 can reduce the cutaneous Arthus reaction, an immune complexes-mediated disease that can result in edema, hemorrhage and neutrophil recruitment in the skin (*36*) (Table 1).

Using mice, Reeve VE *et al.* found that a sub-erythemogenic dose of UVA exposure could protect skin from the immunosuppressive effect of either UVB radiation or cis-urocanic acid (UCA). They indicated that the mechanism of UVA immunoprotection involves the induction of cutaneous HO-1 (*37,38*). Moreover, they reported that UVA irradiation upregulated HO-1 in the dermis and epidermis of hairless mouse skin, and UVA-induced HO enzyme activity is protective against UVB-induced immunosuppression (*10,39*). Further, they demonstrated that, with a constant UVB dose, UVA enrichment showed to be dose dependent for immunoprotection (*10,40*). In their studies, through an HO inhibitor (tin protoporphyrin-IX), it was confirmed that HO is responsible for UVA-induced protection (*10,40*). Also, the refractoriness of UVA-induced protection from photoimmunosuppression was found to be correlated with HO-1 levels with repeated UVA exposure in Skh:HR-2 mice (*41*). The refractoriness of HO-1 was originally found in human skin fibroblast FEK4 cells following retreatment with a second dose of UVA radiation (*42*). Studies from our lab indicate that refractoriness of HO-1 might be linked to UVA-induced Nrf2 refractoriness in human skin fibroblast FEK4 cells (Zhong, unpublished data).

The immunoprotection of HO-1 is closely related to certain cytokines. The immunoprotection of UVA-induced HO-1 was found to be absent in IFN-γ knockout mouse, indicating that IFN-γ is involved in HO-1 induction (*43*). They also indicated that HO-1 could provide protection through its enzymatic product, CO. Utilizing a CO-releasing molecule to deliver CO to the skin, they found that CO concentration-dependently protected mice against the immunosuppression caused by solar simulated UV radiation and cis-UCA (*44*). Further studies indicated that UVA immunoprotection was linked to skin cyclic guanosine monophosphate (cGMP), which is activated by CO. The release of CO is due to HO-1 activation, and this suggests that HO-1 plays a role in UVA-induced immunoprotection (*22*). Moreover, the relationship between HO-1 inducibility and estrogen receptor-β (Er-β) signaling was investigated. They found that the HO-1 gene was unresponsive to UVA induction in Er-β-/- mice, and HO-1 inducibility and Er-β signaling are interdependent requisite responses to the UVA waveband for its immunoprotection (*9*). Recently, they showed that IL-6 has an important photoimmunoprotective function through interaction with the HO-1 pathway, determining the immunologically advantageous actions of UVA radiation (*14*). These studies indicated the relevant pathway of HO-1 in the UVA-induced immune response, and will provide a guideline for more in depth understanding of the mechanism of HO-1 in UVA-induced immunoprotection.

4. UVA Phototherapy

Phototherapy using UVA has been successfully applied to the treatment of some cutaneous diseases, such as psoriasis, vitiligo and T-cell lymphoma (*45,46*). UVA1

Table 1. Evidence of HO-1 in UVA-induced immunoprotection

Experimental subjects	Methods	Involved factors	*Ref.*
SHR1 and C57BL⁄6	CHS	HO-1 and Er-β	(*9*)
C57BL⁄6	CHS	IL-6 and HO-1.	(*14*)
SHR1	CHS	cGMP	(*22*)
SHR1	CHS	UVA-induced HO activity	(*38*)
SHR1, C57BL⁄6, and IFN-γ-/-	--	IFN-γ and HO-1	(*43*)
SHR1	CHS	CO released by HO activity	(*44*)

SHR: Skh:HR hairless mice; C57BL/6: C57BL/6 mice; IFN-γ -/-: IFN-γ -/- mice; CHS: Contact hypersensitivity; MED: Minimal erythema doses.

has been shown to be more effective due to its deeper penetration into skin and less side effects, such as erythema, immunosuppression and carcinogenesis than UVA2. UVA1 can induce apoptosis of skin-infiltrating T cells, lead to T-cell depletion, and induce collagenase-1 expression in human dermal fibroblasts (*47*). These are thought to underlie the UVA1 therapeutics in several diseases, such as atopic dermatitis, inflammatory morphea and scleroderma (*46*). Several studies have also shown the effect of UVA1 on cytokine production. It suppresses proinflammatory cytokines such as TNF-α and IL-12 (*46*). It was also found that UVA1 can exert its beneficial effects in treating systemic lupus erythematosus (SLE) though decreasing IFN-γ, which has a pathogenic role in the development of SLE (*48*). Since UVA can provide immunoprotection *via* induction of HO-1 (long wave UVA is more effective in inducing HO-1 expression when compared with short wave UVA, Zhong, unpublished), we suggest that it would be more effective in treating skin diseases combining HO-1 with UVA1 in phototherapy. Obviously, prior to using HO-1 for the enhancement of UVA phototherapy, mechanisms of immunoprotection linked to HO-1 induction need to be further investigated, as suggested in the literature (*2,45,46*). With the development of technology, using a narrow wavelength range or a mono wavelength of UVA phototherapy will become an efficient method in the treatment of localized and systemic skin disorders (*49*).

5. Conclusions

UVA irradiation may cause both beneficial (immunoprotection) and damaging (immunosuppression) effects on skin, which depend on wavelength, exposure dose, and UVA sources. In this review, the potential protection of specific UVA wavebands and doses to the skin immune response was briefly summarized. Since immunoprotection might be associated with longer wavelengths of UVA (340-400 nm), using narrow bands of UVA or a mono-wavelength in phototherapy can become more effective and have less side effects than using broad bands of UVA. Also, UVA induces high expression of anti-inflammatory enzyme HO-1, which provides beneficial effects on the protection of skin from UVB-mediated damage. Therefore, it is necessary to further verify the role of HO-1 induction using different wave lengths of UVA, in order to modify HO-1 levels in combination with the specific wave length of UVA1 to enhance the efficiency of treating unpleasant skin conditions. Further, this may provide valuable guidance on skin protection against UV exposure.

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Health Management Information System utilization in Pakistan: Challenges, pitfalls and the way forward

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Summary **Use of data generated through the Health Management Information System (HMIS) in decision making has been facing various challenges ever since its inception in Pakistan. This descriptive qualitative study attempts to explore the perceptions of health managers to identify the status and issues in use of HMIS. Overall 26 managers (all men, ages ranging from 26 to 49 years; selected from federal level (2), provincial (4) and seven selected districts (20) from all four provinces) were interviewed face to face. The respondents identified a number of hurdles resulting in non-use, misuse and disuse of data. These included limited scope of HMIS, dubious data quality, political motives behind demand of data and an element of corruption in data reporting *etc.* A great deal of political and administrative will is required to institutionalize transparency in decision making in health management and HMIS is an important tool for doing so. Appropriate legislation and regulations are needed to create a conducive policy environment that would help in changing the existing decision making culture. The effective use of information requires that besides capacity development of district health managers in understanding and use of data, the higher level decision makers are provided with relevant data timely and in an easily understandable form along with the recommended actions pertinent to this data.**

Keywords: Health Management Information System (HMIS), use of information, health managers' perceptions, Pakistan

1. Introduction

A Health Management Information System (HMIS) is meant to provide reliable information to managers at various levels of the health system in a timely manner. It supports decision making in the areas of policy, planning, management, monitoring and evaluation of health systems including it's programs and services.

There could be several barriers contributing to underutilization of data. For instance, inappropriateness of collected data, data gatherers' inability to analyze, interpret and present data/information to decision makers and decision makers' uncertainty on how best to utilize the available data, credibility of information, 'information overload', *etc.* (*1-3*).

A number of non-informational factors can influence the information-based decision making process. These factors might include political views, peers' advice, budget constraints, donors' involvement, unions, community, religious groups, media, special interest groups, *etc.* (*4*).

Use of information for decision making has remained a challenge throughout the evolution of health information systems in Pakistan. Initially due to its inadequacy, the information generated through National HMIS was not helpful either to health managers for system planning or management, or for the health workers for facility or patient management (*5*). The national feedback reports of National HMIS indicated that more emphasis was laid on information collection for the sake of doing it instead of "information for action" (*6-8*). Instead of being utilized at the level

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of generation, data is usually transmitted straight to provincial or National HMIS Units without even being checked (*9*). The reports recommend improving the pertinence and quality of information and timely analyses and quick feedback to the system to ensure timely action. Research pointed out that being irrelevant, unreliable, incomplete and untimely, the data was not helpful for decision-makers. It was pointed out that driving forces behind data or information generation were the donor agencies (*10*). Ministry of Health (MOH) remarked that within a culture of non-evidence-based decision making, planning and management decisions are mostly taken without relevant information and 'making use of information for decision making is discretely practiced' (*3*).

The district health system conceived under devolution reforms, enacted in 2001 in Pakistan, posed another challenge. The literature suggests that the decision-making culture of district government can be based around informal, political information rather than formal, rational information (*11*) and there can be conflict of interest between political and administrative leadership when it comes to information based decision making (*12*). In 2007 a study reported that use of information for management decision making remained uncommon due to limited capacity of managers to understand and utilize data and the lack of accountability and therefore motivation to improve results (*13*).

Since overlooking stakeholders' perceptions is an important reason for information systems failure (*3*), taking stock of the views of health managers is important. Given this backdrop this study was carried out to explore health managers' perspectives about the reasons into and seek their suggestions for an improved use of National HMIS.

2. Materials and Methods

For the purpose of exploring the perceptions of health managers, a descriptive qualitative design was adopted because a qualitative study allows getting close to the people and situations being studied (*14*). Data collection was accomplished through long interviews, therefore data collection tools comprised of in-depth, face to face and semi structured interviews. For this purpose an interview guide was developed to provide an outline on a set of issues which was used as a checklist to capture some of the aspects that might have been eluded otherwise. The questions in the interview guide were not taken in any particular order. Primary data collection was done during September 2004. Overall 26 managers (5 from federal level, 7 from provincial, 8 from selected districts and 6 from health facility levels) were interviewed. The manager was defined as a person with a basic pay scale (BPS) 17 and above, involved in HMIS activities. All respondents were male and were 26 to 49 years old. The respondents included federal level HMIS and vertical program managers, provincial HMIS managers, district health officers, district level HMIS focal persons and health facility charges involved in data management and decision making. In order to collect data, the first author visited the capital Islamabad and six districts from all four provinces (Hyderabad, Lahore, Sheikhupura, Peshawar, Quetta and Mastung). The selection of the districts was based on convenience for the first author in terms of proximity and easy accessibility (*15*). The authors had no power relations with the respondents as they came from independent non government institutions.

Prior to visiting, the respondents were contacted and briefed about the background and purpose of the study and its methodology. After getting consent from the respondents, open-ended questions along with probes were asked during these face-to-face interviews.

It was planned to record the interviews on cassettes, with permission of the respondent and then to transcribe the answers. But in reality only few of the respondents allowed for recording of their interviews. It was also noticed that among the respondents who were interviewed, some had a totally different tone and expression to their normal selves during the tape recording of interviews. This was confirmed by the fact that after the interviews, when the tape recorder was turned off, for example during tea break or before departure, these respondents really spoke out in a drastically different and friendly manner. On enquiring, some of them shared a fear of any potential threat to their position or of being victimized in case the recorded cassette was accessed by some authority against whose performance the respondent had commented freely. Another reason given by a respondent was that he was giving a false picture of HMIS because it was not, in his opinion, a forum to criticize his bosses, the higher management.

So the first author had to change the strategy for recording the responses and took manual notes which had the limitation of interruption during the process of interviewing (*16*).

Data was managed through immediate transcription of responses subsequently after interviews. Analysis was done through categorization of verbatim notes into themes and a general description of the experience that emerged out of statements (*3,17*).

As had been expected, most of the responses were mixed, in Urdu and English. The responses were transcribed in Roman Urdu in a MS Word version. All twenty-six interviews were coded with English alphabet letters (each interview was assigned one alphabet letter) and the page numbers were designated through the same alphabet letter and Arabic numbers. Afterwards line by line analysis was done. Roman numerals were used to indicate the important points in the statement. For example G3-IV meant that on the third page of

the interview assigned alphabet letter G, point number IV is being referred to. Two Filip charts were used on which major themes (drawn from the objectives of the study) and sub themes that emerged out from the responses were noted. At the end the major themes and sub themes of the responses were documented in a long description.

Since data was collected through multiple sources (from various levels of hierarchy as well as from people currently within the program and those key informants, who are currently out of the program), it gave an opportunity to triangulate the findings as well as the contrasts. This type of triangulation is called environmental triangulation because it involves the use of different locations, settings and other key factors related to the environment (*18*).

In addition to this, another form of triangulation was done through peer review by health systems and qualitative research specialists with health sector background.

To be aware of the biases at all stages of inquiry the first author articulated his biases in writing. This bracketing exercise helped to identify and segregate the first author's own perceptions from those of the respondents. Bracketing was employed at each stage of inquiry, from data collection to manuscript writing. A self reflective journal was maintained concomitantly to keep track of day to day events encountered during the research process and record of the feelings, emotions and perceptions of the first author that emerged during the course of recording the interviews. The first author stated his perceptions about the phenomenon being studied before data analysis. This helped as well in understanding the phenomenon from the perceptions of the respondents without a minimum muddling of influence over the data analysis.

Since these were qualitative interviews for which a whole narration was required hence there was a necessity for recording the whole interview. Nevertheless it was anticipated that certain government officials may be either reluctant to have the interview taped or refuse to participate or otherwise may conceal their true perceptions. Given their busy day to day schedule, as was apprehended, some managers were not available or were at outstation at the time of the visit of the first author. Last but not least, given the time and finance constraints, rapport building and gaining confidence of the respondents had become rather difficult.

Before commencement of the interview the first author shared the consent form with the respondent. Through this form the participants were apprised of aims and objectives of the study and the right to refuse to participate at all as well as the right to withdraw during the interview. They were informed that their responses would be recorded on tape (subject to their consent) as well as through note taking. They were given assurance of the confidentiality in the consent form. A written consent was requested but in case a respondent was not comfortable with it, it was not insisted upon. As expected the government employees were reluctant to give anything in writing while they shared their personal experiences and impressions regarding current HMIS to facilitate the communication process. The verbal consent was found to be a more appropriate alternative. A copy of the consent form was retained by both parties and contacts were exchanged for future correspondence. Individual information was kept strictly confidential and the actual responses are quoted without indication of the name of the respondent. It was made clear to the respondents that they might not benefit directly from their participation in the study, as this is neither the objective nor the scope of this study.

3. Results

The following themes emerged from the responses:

3.1. *Potential venues for HMIS utilization*

The respondents enumerated a number of potential uses of information generated by HMIS, ranging from developing a vision and policy formulation to short and long term planning, needs assessment, better targeting and managing day to day affairs. Categorically mentioned areas were assessment of staff performance, quality and utilization of services, measures of diseases and their patterns, budgeting and financing, program monitoring, and comparison of health facilities. It was iterated that HMIS could be used as a check to corruption/pilferage, as a tool for prevention (*e.g.* early warning of epidemics), as a means to justified demand for resources, resources distribution/redistribution/ allocation as per principles of equality, equity, or as per disease patterns.

> '*HMIS can give a clue of many visible and hidden aspects of the health sector and can be used for research purposes.*' *(Provincial level manager)*

3.2. *Perceptions on current utilization of information*

3.2.1. *Policies, planning and management*

Respondents deemed policy making in Pakistan a '*haphazard process*' with little spade work in its formulation. They mentioned '*ad hoc policy formulation*' and presence of '*unsubscribed policies*' as an acceptable norm in the culture, as opposed to an evidence-based policy. Likewise they perceived that while planning, situational analysis is hardly ever undertaken and HMIS generated data is not given consideration.

'*If a planner at a higher level wants to establish a twenty-bed hospital, he asks the DHO* [District Health Officer] *to make a feasibility study* [report] *within two days.*' *(Provincial level manager)*

Occurrences of emergencies were cited in the context of planning.

'*We plan the day when we learn that, say, an outbreak of gastroenteritis has taken a large toll; we then immediately send a team to respond to the situation and in the end we report* [that] *the situation is under control.*' *(Provincial level manager)*

Higher management can also demand data only occasionally:

'*If tomorrow is a meeting then DG* [Director General of Health services] *will ask today about the number of TB patients. Otherwise he is least bothered for data.*' *(Provincial level manager)*

3.2.2. *Motives behind demand for human resources related data*

If by any chance the data is demanded, it is for personal motives.

'*The minister enquired about vacancies in a certain area. We realized later that he intended to get some of his people appointed.*' *(Provincial level manager)*

A personnel related record is a much sought after entity from the Health Department. However,

'*The motive is rarely to assess the human resource status of health facilities, so as to take corrective measures e.g. transfer staff from an underutilized facility to one experiencing a high patient load owing to staff shortage.*' *(District level manager)*

3.2.3. *HMIS reports: A supply without a demand*

Equating HMIS with a '*data warehouse*' and '*junkyard*' for the '*dumping*' of reports, respondents perceived that the purpose of the data generation activity is nothing but to serve the purpose of printing a report.

'*They just publish a book* [annual HMIS report] *… no one does the follow up of the report … there is no use for this report, even when the report is published, as the time lag is too large.*' *(Provincial level manager)*

3.2.4. *Non use of HMIS results in incongruous resource distribution*

Referring to inappropriate distribution of resources, a district level health manager submitted:

'*Under certain donor supported projects, various equipment such as four anesthesia machines got dumped due to un-informed management and the donor agencies. The distressing part of the story is that there was no trained operator available in the same facility while in a nearby district there was a trained operator who had no machine with him. All this was the result of non use of HMIS.*' *(District level manager)*

3.2.5. *One way traffic: Yearn for feedback and compliance*

Health facilities reported a chronic problem with the supply of medicine which is provided on a push system basis (*i.e.*, a fixed package is delivered regularly) for each facility. They reported and demanded a certain type and quantity of medicines based on the data of their facility but in response the same package of medicine is delivered always.

'*No evidence-based adjustments are done for sending required medicines on our monthly reports.*' *(District level manager)*

'*Since resource allocation is not appropriate and need based, it is likely that at one facility there would be permanent shortage of a certain medicine and at the other there will be loads of the same medicines expiring on account of no need.*' *(Health facility manager)*

3.2.6. *Culture of decision-making*

Respondents indicated that at the federal level certain '*directives*' exist under which policy formulation related strategic decisions are made. The lower tiers of management at provincial levels are essentially implementers and planners.

The term "adhocism" was frequently iterated while describing the culture of decision making. Adhocism was equated with shortsightedness, patchy solutions and being *not bothered by the future*.

'*During twenty years of my service, I noticed health managers' short sightedness; they think of 'today' only, future planning is rarely done. No thought of the coming fifty years. This is the adhocism.*' *(Provincial level manager)*

3.3. *Perceived hurdles in use of HMIS data in decision making*

3.3.1. *Limited scope of HMIS limits its potential usefulness*

HMIS is providing outpatient department (OPD) based data and not catering to a number of other important functions being performed and services being delivered by the health department. Owing to this fact that HMIS communicates information for only a very small portion of the health care sector in the country, it has minimal

utility for policy makers.

A number of public sector First Level health Care Facilities (FLCFs) are underutilized as the majority of patients bypass these and get direct access to the secondary and tertiary level public sector health care facilities.

'*The major load* (of patients) *is at the level of Tehsil Head Quarter Hospital (THQ), District Head Quarter Hospital (DHQ), and at teaching hospitals but* (the) *HMIS does not cover these* (institutions).' *(Federal level manager)*

In addition, a number of facilities are not functional and even those that are functional do not regularly report (National Feed Back Report 2006, National Health Management Information System 2006 indicates that out of 11,732 FLCFs, the number of functional FLCFs was 9,351 and out of these approximately 8,000 were sending reports.).

Furthermore, while the majority of the patient load is towards the private sector (According to Pakistan Social and Living Standards Measurement Survey (PSLM) 2008-09, the use of the private sector for outpatient consultations has risen from 69 percent in the late nineties to 79 percent in 2006-2007.), HMIS is not capturing this majority.

Hence the information generated through HMIS does not reflect the actual health status of the masses and therefore has limited utility for policy makers (Figure 1).

District level managers felt a dearth of information on in-patients, financial management, vacancies' status, training status and needs of various cadres in the health sector in HMIS.

In certain districts, refugees from Afghanistan are major beneficiaries and account for a considerable patient load at public sector health facilities yet HMIS does not speak on this.

Other areas not addressed in HMIS include medico legal type cases (including gender based violence) and quality of care. A few respondents reflected that HMIS lacks disaggregated information on asymmetries such as cause specific mortality and morbidity among various socio economic strata that may indicate the disparities between gender, poor and rich and hence does not offer support for minimization of urban bias.

'*HMIS is silent about the health problems of various classes and ethnic groups, their deprivation and poverty status.*' *(Federal level manager)*

3.3.2. *Vertical programs: Parallel reporting*

As the HMIS depicted picture is not in tune with 'Burden of Disease' (BOD) estimates, considered a more reliable measure, separate, vertical information systems were introduced for rapid information transmission for each of these vertical programs. The failure of HMIS to provide the necessary information channel for these vertical programs resulted in generation of two reports pertaining to the same activity. One report is sent to the district health office and the other is sent to the concerned program manager who sends that directly to federal authorities. These two reports show different figures which creates confusion.

At the district level managers felt overburdened by producing multiple reports since each vertical program demands its own report. This indicates the lack of coordination between various information systems, duplication of efforts and resource wastage.

3.3.3. *Reliability of data creates hesitancy among potential users*

Some of the issues surrounding the questionable quality of data, as narrated by the respondents, are summarized in Figure 2 which indicates that right from the health facility level poor data entry leads to inconsistencies up to the top level.

At provincial HMIS cells the respondents complained that other concerned provincial departments never demand data nor valued it. Apparently the Planning Cell doesn't need it or has doubts about the reliability and timeliness of data.

At the facility level, non-use of data was correlated to lack of staff's capacity to use the information. It was said that they can't understand simple graphical data displays and cannot perform simple calculations. Besides this some other reasons perceived from non-use of information at various levels are summarized in Figure 3.

Some respondents asserted that being fictitious, it was of no use to utilize data or these reports. They revealed, for instance, that during visits they could judge that inaccuracies due to careless data entry were evident from the total number of certain cases given treatment that did not tally with "days out of stock" status of the relevant medicine.

3.3.4. *Bogus reporting and "data creation"*

According to a provincial manager:

'*The FLCF has no direct contact with the community, as the Lady Health Work* (LHW) *has, so how is it possible for FLCF to report on IMR, when the infant death has occurred, say, four miles away in the community… Similarly doubts about reliability of the repots arose when it was noticed that some districts kept on reporting cases of guinea worm that was long been eradicated.*' *(Provincial level manager)*

A health facility level manager admitted:

'*Whatever we are doing is not fair… At the time of closing of the monthly register, looking at the*

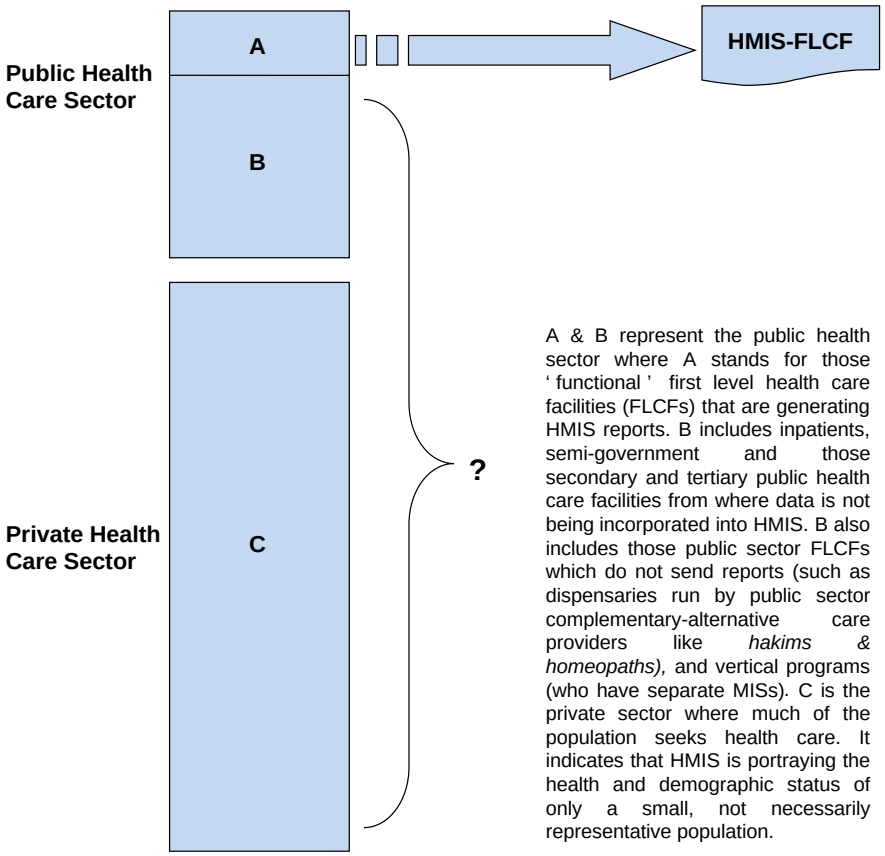

Figure 1. What HMIS Actually Portrays.

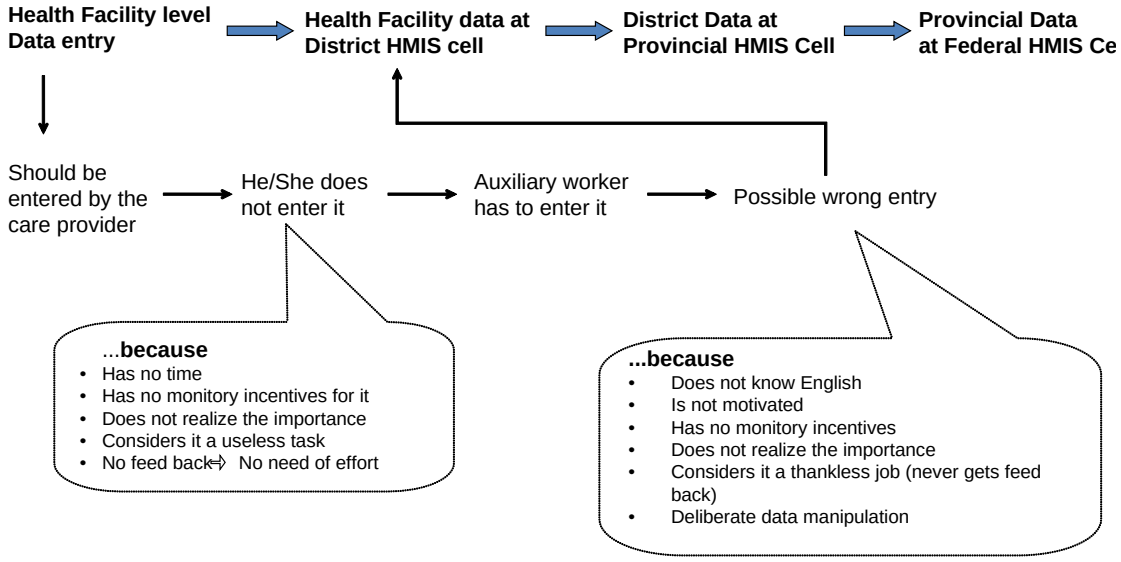

Figure 2. How data quality deteriorates.

gender distribution, considering the seasonal patterns and keeping priority diseases in front, an abstract is written down. If there is summer we report diarrhea and if winter more acute respiratory infections (ARI) cases… So no one will inquire.' *(Health facility level manager)*

Respondents at MOH maintained that it was indeed the busy routine administrative life of the managers that doesn't allow them to spare sometime "*for any technical issue such as Disease Early Warning System (DEWS), the disease profile, epidemic management, coverage plan, some kind of calculations, etc.*" To others, unavailability of support staff could be another reason:

'*Getting entangled in numbers and figures is a*

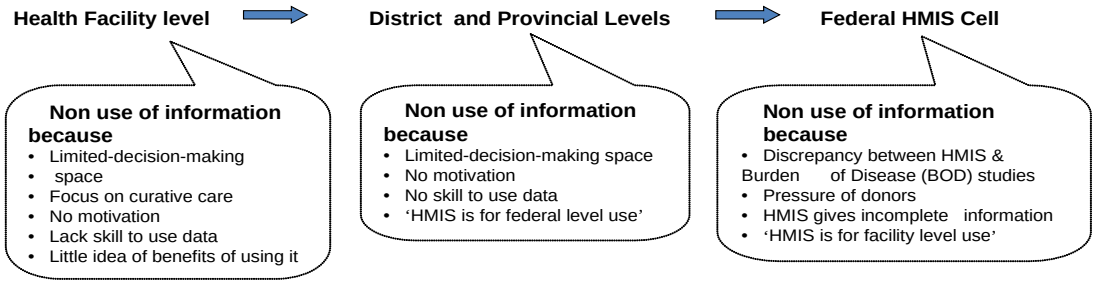

Figure 3. Why information is not being used at various levels.

difficult task, particularly if you don't have staff to support you in this.' (Facility level manager)

3.3.5. *Data manipulation*

Besides calling careless data entry an act of "just doing away with routine work", it was reflected that data is manipulated for some ulterior motives as well. For example referring to the polio campaign it was said that some area supervisors will indicate a falsely increased targeted population to get more and more funds. The deliberate data manipulation could be categorized under two headings. First is data manipulation for personal motives, and second for material gains. As in the words of a respondent:

'*The basic health unit is an earning spot. If two rupees per chit is charged per patient, then there would be 2,000 rupees collected from 1,000 patients. But if only 500 patients are entered in the record, 1,000 rupees can be kept in the pocket… Similarly a patient can get* [a whole course of] *tablets by paying 5 rupees extra otherwise he or she may be given a single injection and would not be attended further.' (District level manager)*

This was the artificial regression of patient turnover. The second category of data manipulation could be artificially inflating patient turnover. This could further be due to two reasons. Either there is strictness from the concerned authority that there must be more patients than a certain number, or be seen, at the health facility otherwise that facility might be considered underutilized or inefficient (in certain areas it was shared that health facilities where the patient turnover was less than five hundred, in punishment, their salary was withheld). Or the purpose of artificially inflating could be to ensure that medicines could be obtained as per quota or even more than the quota. Certain facilities are given a quota for one quarter Rs. 3,000 (1 USD = 85 Pak. Rs) to 5,000 worth of medicine. This comes to be around fifteen hundred rupees worth of medicine per month. And if 50 rupees worth of medicine is given to each patient, only 20 to 30 patients per month can be entertained while the patient load is usually much more than that. Thus the facility managers show more patients than they actually see so that if not more, then at least as per quota the medicine can be obtained because if they show the correct number of patients attended, they fear that they will be given medicine below the fixed quota.

4. How can HMIS utilization be improved

Despite aforementioned reservations about HMIS utilization the managers also shared their suggestions for the improved utilization of HMIS. (Parenthesis indicate the number of respondents suggestions came from)

i) At the policy level the leadership commitment could play a very important role in the use of information generated through HMIS (9).
If policy makers start demanding data and information, there would be a trickledown effect. It is necessary to utilize the present HMIS as it is as, for, '*the more there is demand for the data the more it becomes refined over time*'.

ii) HMIS data transmission should be brisk and the feedback should be forwarded to the highest level of management (24) (including '*secretary of health and director of health services*').

iii) Regular and timely feedback to facilities may encourage managers to utilize the information at local levels as well as build capacity of the local managers and provid them some incentive (such as further training) which may help improve quality of services (19).

iv) Introducing an environment of competition among health managers of different districts could be a beginning of use of data. If in a monthly meeting the managers of various facilities are joined and the trends and patterns of disease are compared and contrasted, this may generate a competitive environment for the display of data and an urge to take practical measures to solve problems and improve indicators (8).

v) Use of HMIS at the Community level is of vital importance. Community based LHWs can play an important role, as they have good rapport with the women groups, health committees and functional CBOs in the area. LHW can

disseminate important and pertinent information about certain health care matters and also important health indicators such as IMR (13).

vi) Vertical information systems should be integrated into one central HMIS system, in order to reduce duplication of work and the ambiguity it creates among users of health information (16).

vii) The scope of HMIS should be increased. If more information such as in-patient related data, private sector coverage, financial management, *etc*. is added to the current HMIS, it would become more useable (6).

5. Discussion

Be the use of information on managerial grounds or from politico economic perspectives or even on ethical and rights based concepts, it is important to consider the context or intention under which information is collected and the context in which the collected information is analyzed.

Respondents spoke of corruption in its various forms. They mentioned it in terms of non use, abuse and misuse of data and information. They suggest one solution for the problem was enhancing monitoring and supervision and others opined that transparency and accountability could be a way out of the problem. Most considered corruption to be preoccupying the whole system from top to bottom.

Respondents indicated multiple constraints in decision making at their levels including limited choices, scarcity of resources, pressure from top management and coercion by the outside agencies (local political and donor agencies).

Political interference is by far the strongest factor that interferes with the well-functioning of the system and rational use of information. Under the devolution reforms this appears to be a very challenging job. The political leadership is perceived to come with a different agenda or ulterior motive that may not necessarily build on the information system as a requirement. Advocacy channels might be required to create a culture of use of information in decision making.

Expectations from the system also need clarity as these are directly related to ownership of the system. One needs to be educated to get a clear idea about what HMIS can do and what it can't. On the one hand there is an expectation of such a comprehensive system that encompasses each and every aspect directly or indirectly related to health. On the other hand there is the system that is being run for the sake of running it; where its movers and shakers perceive it as a task being performed for someone else. Despite the very idea of HMIS being a tool primarily meant to be used by the managers at the facility and district level, an idea promulgated since the very beginning of the system by the MOH, the perceptions at various levels persist that HMIS is meant for the higher or the lower levels. The use of HMIS needs to be demonstrated at various levels at least in some model that can be shared with the other stakeholders.

A great hurdle has been seen in the integration of HMIS with the vertical programs. Literature suggests that this problem even exists in the developed world as well where the donor driven vertical system, manpower and management hierarchy, distinguished from the line management, makes routine information systems chaotic (*4*). Some of our respondents suggested coordination as a solution to this problem. But the experts recommend a uniform system and not "better coordination" as a solution to duplication and waste problems seen in parallel health information systems.

Data entry was not considered an important task and fudged data has reportedly been entered just to generate the report *per se*. Creating demand for data might be brought in through a transparent system where the decision makers are asked about the way they decide about certain managerial functions. If a decision maker is answerable to some learned people with a given protocol of decision making then it might be possible to incorporate the evidence base in making decisions.

In this study only one type of stakeholders has been covered. Under the devolution reforms the number of decision makers at the district level has increased. We recommend exploring the views of such potential users/ stakeholders for the information generated through HMIS.

6. Conclusions

Improved decision making requires institutionalization of a dialogue between data generators and the decision makers and those who influence the decision makers. Timely dissemination of relevant data to decision makers in an easily understood form along with the recommended actions based on this data is a prerequisite for effective use of information. A great deal of political and administrative will is required to institutionalize transparency in decision making and HMIS is one of the means towards that. For development of systems for the collection, collation, communication, and use of health information from the un-captured areas of public and private health care sectors, government is required to create a conducive policy environment through legislation and regulation.

District managers need to have the basic skills for day to day decision-making using information generated through HMIS. They need to build their skills for creating a supportive environment for the improvement of data quality and the use of evidence-based management.

Instead of being limited to the mere description of health problems, it would be of great value for decision-makers to receive interpretation of raw data

and evidence-based recommendations with appropriate actions and alternative solutions for alleviating and phasing out problems. Furthermore, to ensure quality and transparency, it would be necessary to incorporate elements to evaluate health interventions and public health programs based on a well operating HMIS.

7. Interview Guide

The interview was guided as follows:

- What in your opinion is the importance of HMIS?
- What are the weaknesses/limitations in the current HMIS?
  - o Why do these exist?
  - o How would you suggest bringing improvements in these?
- What do you know about evidence based decision making (decisions based on some valid information)?
  - o What is the role of HMIS in evidence based decision making?
- What type of information do you need (in your managerial capacity)?
  - o How would you comment whether the same information you mentioned is provided to you by the HMIS or not?
  - o Please suggest how these needs can be fulfilled.
- Are there any hurdles in the use of information generated by HMIS?
  - o How can these be overcome?
- What in your opinion is the role of HMIS in new decentralized system/under post devolution reforms?
  - o Are there any hurdles in use of HMIS under decentralized system?

8. **Postscript**

This is the first study ever specifically focusing on the use of HMIS generated information in Pakistan. It documents some of the very "unspoken" and "untouched issues" in the health systems literature. The significance of this qualitative enquiry remains for those who are interested in studying the impact of devolution on the culture of decision making.

On 1st July 2011, the Federal Ministry of Health of Pakistan was completely devolved in compliance with the 18th Constitutional Amendment. The National HMIS developed in the 1990s became defunct. Prior to the 18th amendment, planning and implementation of health programs rested with the provinces whereas the federal government had a stewardship role to play to improve the health status of the general population. In order to fulfill its stewardship role the Federal Ministry of Health remained responsible for formulating and setting national policy guidelines. Before the devolution of the Federal Ministry of Health (2011) the last draft National Health Policy of Pakistan that appeared in 2009 clearly indicated that monitoring remains weak at all levels due to an absence of a result based culture. It stated that although HMIS was functional but due to compromised data, quality use of information for decision making was discretely being practiced.

The Harvard study (2007) commissioned in some districts of Pakistan showed that improvements can be achieved in a decentralized system characterized by a broader scope for decision making, better capacity, and greater accountability of officials to local authority (*19*). The devolution can take stock of similar evidence at this crossroad as the provinces are now fully empowered under a democratic government to devise their province specific policy and strategic framework and make policy decisions. This paper makes a case for the provinces to fulfill the legal requirement of employing evidence-based decision making. This is a high time for the provinces to inculcate a culture of transparency and evidence-based decision making. The lessons learned at the Federal level can be utilized at the Provincial level at this very early stage offer new development.

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High rate of unintended pregnancies after knowing of HIV infection among HIV positive women under antiretroviral treatment in Kigali, Rwanda

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Summary **More than 90% of pediatric AIDS results from the transmission of the virus through HIV positive pregnant mothers to their children. However, little has been known about factors associated with unintended pregnancies after knowing their HIV seropositive status, or contraceptive use among HIV positive women under antiretroviral treatment (ART). We investigated thus factors associated with unintended pregnancies after knowing seropositive status, and also factors associated with the non-use of contraceptives among HIV positive women under ART. We carried out a cross-sectional study in Kigali, Rwanda in 2007. A total of 565 HIV positive women under ART were interviewed. We examined the associated factors of unintended pregnancies or non-use of contraceptives using logistic regression analysis. Among all the respondents (*n* = 565), 132 women became pregnant after knowing their HIV seropositive status. Among them, 82 (62.7%) got pregnant unintentionally. Those who had two or more children (adjusted OR, 3.83) were more likely to get pregnant unintentionally. Meanwhile, among all, 263 had sexual intercourse during the last three months. Of them, 85 women did not use any contraceptives. Those who did not agree that 'HIV positive children can survive as long as HIV negative children' (adjusted OR, 2.28), and those who 'can always ask partner to use a condom' (adjusted OR, 9.83), were more likely to use contraceptives. This study suggests that HIV positive women under ART need special support to avoid unintended pregnancies especially those who have two or more children. Moreover, interventions are also needed to improve women's understanding of the prognosis of pediatric AIDS, and condom-use negotiation skills.**

Keywords: HIV/AIDS, mother-to-child transmission, contraceptive, unintended pregnancy, Rwanda

1. Introduction

Approximately 33 million people were living with HIV/AIDS in the world in 2008 (*1*). Although HIV prevalence seemed to be globally stabilized in 2007, Sub-Saharan Africa still remains the hardest hit area (*2*). Rwanda is a country with a 9.92 million population (2008), and its gross national income per capita was 410 US dollars in 2008 (*3*). Total fertility rate was 5.9 in 2007, and the contraceptive prevalence was 17.4% between 2000 and 2006 (*4*). Available contraceptives are the pill, injection, condoms, IUD, norplant, and sterilization (*5*). As for the statistics related to HIV/AIDS, HIV prevalence among the 15-49 years old population was estimated at around 2.8% [2.4-3.2%] in 2007 (*6*). HIV prevalence among 15-24 years old pregnant women was 9.8% in 2001 in Kigali, the capital city of Rwanda (*5*). HIV prevalence in 0-14 year olds accounted for 14% of the total number of people living

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with HIV/AIDS in Rwanda (*6*).

More than 90% of pediatric AIDS results from the transmission of HIV from HIV positive women to their children (*2*). Thus, unintended pregnancy of HIV positive women who know their seropositive status is considered one of the most important issues to be addressed immediately to prevent transmission of the virus (*7*). The prevention of unintended pregnancies among HIV positive women is emphasized in the four-pronged strategy for preventing mother-to-child transmission of HIV developed by the United Nations. In addition, it has been reported to be a more essential and more cost effective approach to reduce pediatric AIDS than use of antiretroviral drugs for HIV positive pregnant women (*8*). Nevertheless, the rate of unintended pregnancies after knowing HIV seropositive status is considerable in some countries. In Cote d'Ivoire, for example, around 50% of pregnancies that occurred after knowing of their HIV seropositive status were unintended (*9*). In Uganda, more than 93% of women under ART who got pregnant during the follow-up period, expressed not wanting or not planning to have more children (*10*). While these studies indicated the importance of preventing unintended pregnancies, they did not explore its related factors. Such information would be useful to design specific interventions that aim to reduce unintended pregnancies among this population. However, only a few studies have been conducted on this important issue.

Accordingly, using contraceptives is one of the important practices for HIV positive women to prevent unintended pregnancies. For this, we also need to identify factors associated with the non-use of contraceptives among HIV positive women. Previous studies have reported several factors that were associated with the non-use of contraceptives among HIV positive women. Such factors included: no formal education, desire to have children, not knowing the prevention of mother-to-child transmission (PMTCT) program (*10*), religion, infertility (*11*), a lack of trust in condoms as protection, and the male partner's refusal to use condoms (*12*). However, little has been known about the relationship between the non-use of contraceptives and perception of mother-to-child transmission (MTCT) such as prognosis of pediatric AIDS, or self-efficacy of condom use.

The objective of this study was to investigate the factors associated with unintended pregnancies or the non-use of contraceptives after knowing of seropositive status among HIV positive women under ART, to which an increase of pediatric AIDS would contribute.

2. Methods

2.1. *Study design and site*

We conducted a cross sectional study from 25th July to 17th August in 2007 in two health centers, Kinyinya and Kimironko in Kigali. In these two centers, Médecins Sans Frontières (MSF) Belgium intervened from 2002 to 2007 and scaled up ART for HIV positive people. Since the beginning of the ART program in 2002, 2,039 patients have started ART through these two centers as of 2006.

2.2. *Participants*

The participants of this study were HIV positive women under ART, who visited the two health centers during the study period. Inclusion criteria were women aged between 20-49 years old who were not pregnant on the interview day, to determine past pregnancies post HIV diagnosis. A total of 565 women attending the clinics during the study period met the inclusion criteria and we recruited all of them.

2.3. *Questionnaire survey*

We conducted face-to-face interviews using a structured questionnaire, which was developed based on the UNAIDS general population survey (*13*) and other previous studies (*10,14-17*). We developed the questionnaire first in English and then translated it into Kinyarwanda (the official language in Rwanda). It was then translated back into English. After that, we pre-tested the questionnaire on 45 participants and revised it accordingly.

2.4. *Data collection*

We collected socio-demographic data such as age, religion, marital status, job, education, and duration of ART. As for pregnancy related information, we asked participants about their pregnancy experience, the number of living children, history of children's death, past history of pregnancy after knowing of seropositive status (excluding the cases in which seropositive status was detected during the pregnancy), reason and intention of all the pregnancies after knowing of seropositive status, desire to have children, and experiences of receiving counseling. As for reasons of pregnancy, we categorized them as unintended pregnancy and intended pregnancy based on whether the woman's pregnancy was either mistimed or unwanted at the time of conception or not (*18*).

We measured MTCT knowledge using four questions such as, 'whether they know that HIV can be transmitted from mother to child at delivery', 'through breast milk' or 'during pregnancy', and 'whether HIV positive women on ART might deliver an HIV positive child'. We categorized the participants into two groups: those who have a high MTCT knowledge, and others. Those who gave correct answers to all the four questions were categorized as having a 'high

MTCT knowledge' and the others are those who did not give correct answers. To assess their perception of the prognosis of pediatric AIDS, we asked 'whether they agree that HIV positive children can survive as long as HIV negative children'.

To measure the non-use of contraceptives, we first asked the participants about the frequency of their male condom use (never, sometimes, always) in the past three months as it is the most common contraceptive method. For those who answered "sometimes" or "never", we asked whether they had used any other contraceptives in the past three months such as the pill, injection, condoms, IUD, norplant, and sterilization. Then we defined women who did not use any contraceptive methods recently as those who do not use contraceptives. We also asked about the self-efficacy of condom use, the belief that one is capable of asking their partner to use a condom, and its frequency (never, sometimes, and always).

We recruited six qualified nurses as interviewers. The first author gave one-day training to all interviewers and then they undertook a three-day pre-test trial. After the pre-test, they collected data. We conducted the survey in isolated spaces to ensure the personal privacy of respondents. Interviews were completed within 25-30 minutes after their informed consents were obtained. During the survey, the first author supervised interviewers and had a meeting with them to give them feedback. The study was approved by the Ethical Committee of the University of Tokyo (approval number: 1669). We also obtained written permission to conduct this study from the Treatment and Research of AIDS Center (TRAC) in Rwanda.

2.5. *Data analysis*

We examined the associated factors of unintended pregnancies or non-use of contraceptives using logistic regression analysis. In our multivariate analysis, we included all the variables which were significant in bivariate logistic regression analysis at $p < 0.25$ as recommended by Katz (*19*). We also entered other variables which were associated with unintended pregnancies or non-use of contraceptives in previous studies. Statistical significance was set at a $p < 0.05$ level.

3. Results

3.1. *Socio-economic characteristics*

As shown in Table 1, the median age of the participants was 35.8 (S.D. = 6.3) years old. Regarding religion, 307 (54.3%) were Protestant, 162 (28.7%) were Catholic and 22 (3.9%) were Muslim. Among all the respondents (n = 565), 312 (55.2%) were widowed, separated or divorced, and 222 (39.3%) were married or cohabiting. Regarding their job, only 58 (10.3%) had regular or irregular jobs with wages. Those who had formal school education for at least 1 year were 369 (65.3%). The

Table 1. Socio-economic variables of the respondents (n = 565)

Variables	*n*	%
Number of respondents		
Kimironko health center	291	51.5
Kinyinya health center	274	48.5
Socio-economic variables		
Age (Median: 35.8 (S.D. = 6.3) years old)		
< 35	279	49.4
≥ 35	286	50.6
Religion		
Protestant	307	54.3
Catholic	162	28.7
Muslim	22	3.9
Other	74	13.1
Marital status		
Widowed/separated/divorced	312	55.2
Married/cohabiting	222	39.3
Single	31	5.5
Have paid jobs (yes)	58	10.3
Formal education of women* (yes)	369	65.3
Under ART years (Median: 1.9 (S.D. = 1.2) years)		
< 2	228	40.4
≥ 2	329	58.2
Cannot remember	8	1.4
Sero-status related variables		
Partner's sero-status among married and cohabiting women† (n = 222)		
Positive	155	69.8
Negative	43	19.4
Don't know	24	10.8

* Formal education = primary or secondary; † Excluded widowed, separated, divorced, and single, not applicable = 343.

median duration of receiving ART was 1.9 (S.D. = 1.2) years. Among those married and cohabiting participants (n = 222), 155 (69.8%) mentioned that their partner was seropositive.

3.2. *Pregnancy related history*

As for the history of pregnancy in the past (Table 2), 535 (94.7%) had ever been pregnant. The median number of children was 2.5 (S.D. = 1.8), and 273 (48.3%) experienced the death of a child. Of the total, 132 (23.4%) got pregnant after knowing their HIV seropositive status. The median number of these pregnancies was 1.1 (1-5 times). These pregnancies occurred within 4.8 years on average (1-17 years). Of 132 who got pregnant after knowing of seropositive status, 82 (62.1%) got pregnant unintentionally and 112 (84.8%) received PMTCT. The main reasons for unintended pregnancies were: 'their partner did not want to use a condom' (26.8%), 'their partner's desire to have children' (17.1%), and 'a condom was torn' (15.9%). On the contrary, 34 (25.8%) got pregnant intentionally after knowing their seropositive status. The main reasons for intended pregnancies were: 'having no children' (44.1%), and 'had a few children' (20.6%).

Regarding the experience of receiving counseling in any facility after knowing of their seropositive status up until the day of interview, 369 (65.3%) received counseling about 'method of contraception', 324 (57.3%) received counseling about 'how to discuss with partner about family planning', 318 (56.3%) received counseling about 'how to get the contraceptives'. Of the total, 67 (11.9%) desired having more children.

3.3. *Knowledge and perception*

Regarding knowledge of the transmission routes of HIV from mother-to-child (Table 3), 551 (97.5%) knew about delivery and breast-feeding routes, and 473 (83.7%) knew about HIV transmission during pregnancy. However, only 367 (65%) knew that 'ART does not always prevent transmission of HIV'. Of the total, 315 (55.8%) chose all the correct answers. As for the perception, 240 (42.5%) thought that 'HIV positive

Table 2. Pregnancy experience (n = 565)

Variables	n	%
Variables related to pregnancy in her life		
Ever experienced pregnancy (yes)	535	94.7
Number of living children (Median: 2.5 (S.D. = 1.8))		
≥ 2	389	68.8
< 2	176	31.2
Ever experienced the death of a child (yes)	273	48.3
Variables related to pregnancy after knowing of seropositive status		
Ever got pregnant after knowing of seropositive status		
Yes	132	23.4
No	433	76.6
Number of pregnancies after knowing of seropositive status (Median: 1.1 (S.D. = 0.8))		
0	433	76.7
1	97	17.2
≥ 2	35	6.2
Reason of pregnancy after knowing of seropositive status*	132	
Unintended pregnancy (n = 82)		
Partner did not want to use condoms	22	26.8
Partner wanted children	14	17.1
Condom was torn	13	15.9
Did not want children, but did not use any contraceptive method	10	12.2
Did not know how to practice family planning	3	3.7
Rape	1	1.2
Not mentioned	19	23.2
Intended pregnancy (n = 34)		
Had no children	15	44.1
Had only a few children	7	20.6
Desire for male/female children	4	11.8
Desire for non-infected children	3	8.8
Not mentioned	5	14.7
No answer (n = 16)	16	
Received counseling after knowing of sero-positive status on		
HIV transmission from mother to baby through pregnancy	504	89.2
Method of contraceptives	369	65.3
How to discuss with partner about family planning	324	57.3
How to get the contraceptives	318	56.3
Desire to have more children (yes)	67	11.9

* 433 participants who did not have experience of pregnancy after knowing of sero-positive status were excluded. The participants who knew their HIV-positive status during the pregnancy were not included.

Table 3. Knowledge, perception and related behaviors (*n* = 565)

Variables	*n*	%
HIV related knowledge		
Know HIV can be transmitted from mother to baby at delivery	551	97.5
Know HIV can be transmitted from mother to baby through breast milk	551	97.5
Know HIV can be transmitted from mother to baby during pregnancy	473	83.7
Know HIV positive women under ART might deliver HIV positive babies	367	65.0
All correct answers	315	55.8
Not all correct answers	250	44.2
Perception		
Think HIV positive babies can survive as long as HIV negative babies (yes)	240	42.5
Self-efficacy		
Can ask partner to use condoms (*n* = 222)*		
Always	121	54.5
Sometimes	40	18.0
Never	61	27.5
Sexual behavior		
Had sexual intercourse during the last three months (yes)	263	46.5
Used condoms among those who had sexual intercourse during the last three months (*n* = 263)†		
Always	157	59.7
Sometimes or never	106	40.3
Used a contraceptive method among those who did not always use condoms (*n* = 106)‡		
Always	21	19.8
Sometimes or never	85	80.2

* The participants who were widowed, separated, divorced, or single were excluded (*n* = 343); † The participants who did not have sexual intercourse during the last three months were excluded (*n* = 302); ‡ The participants who did not have sexual intercourse during the last three months or who always used condoms were excluded (*n* = 459).

children can survive as long as HIV negative children'.

3.4. *Self-efficacy*

For self-efficacy of condom use among the 222 married or cohabiting women (Table 3), 121 (54.5%) stated that 'they can always ask partner to use a condom' and 40 (18.0%) stated that 'they can sometimes ask partner to use a condom'.

3.5. *Use of contraceptives*

Among all the respondents, 263 (46.5%) had sexual intercourse during the last three months (Table 3). Of the 263, 106 (40.3%) did not use condoms consistently, and of the 106, 85 (80.2%) did not use other contraceptive methods (those who always used at least one contraceptive methods were 178). These 85 participants were categorized as the group of non-users of contraceptives.

3.6. *Factors associated with unintended pregnancy*

Table 4 shows the factors associated with unintended pregnancy among women who had gotten pregnant after knowing their sero-positive status. Among 132 women who got pregnant after knowing their sero-positive status, 116 women who responded with a reason for the pregnancy are the subjects of this table. As the table shows, the multivariate analysis indicated that the participants who have two or more children (adjusted OR, 3.83; 95% CI, 1.30-11.30) were more likely to get pregnant unintentionally after knowing of their seropositive status.

3.7. *Factors associated with contraceptive use*

Table 5 shows the factors associated with contraceptive use among all participants. As this table shows, the multivariate analysis indicated that the participants who did not agree that 'HIV positive children can survive as long as HIV negative children' were more likely to use contraceptives than those who agreed to it (adjusted OR, 2.28; 95% CI, 1.12-4.63). Compared to the participants who can never ask partner to use a condom, those who 'can always ask partner to use a condom' (adjusted OR, 9.83; 95% CI, 4.28-22.61), and 'can sometimes ask partner to use a condom' (adjusted OR, 11.12; 95% CI, 4.89-25.29) were more likely to use contraceptives.

4. Discussion

Our study is one of the few which focused on unintended pregnancies of HIV positive women under ART, one of the urgent issues of HIV/AIDS. Our study revealed that almost two-thirds of HIV positive women pregnancies, after knowing their seropositive status, were unintended, and a considerable number of women who had sexual intercourse during the last three months had not consistently used contraceptives. Our findings also suggested that the number of their living children was significantly associated with their unintended pregnancies. Moreover, we demonstrated

Table 4. Factors associated with unintended pregnancy after knowing of sero-positive status (n = 116)†

Variables	Unintended pregnancy after knowing of seropositive status		Crude OR (95% CI)	Adjusted OR# (95% CI)
	Yes (n = 82) (n (%))	No (n = 34) (n (%))		
Socio-economic variables				
Age (years old)				
≥ 35	27 (79.4)	7 (20.6)	1.89 (0.73-4.90)	1.63 (0.60-4.42)
< 35	55 (67.1)	27 (32.9)		
Religion				
Protestant	42 (70.0)	18 (30.0)	1.99 (0.58-6.86)	2.11 (0.54-8.21)
Catholic	25 (75.8)	8 (24.2)	0.78 (0.08-8.04)	0.66 (0.62-7.09)
Muslim	4 (80.0)	1 (20.0)	1.34 (0.51-3.53)	1.43 (0.52-3.93)
Other	11 (61.1)	7 (38.9)		
Have paid jobs				
Yes	2 (100)	0 (0)	--	--
No	80 (70.2)	34 (29.8)		
Formal education of women				
No	29 (74.4)	10 (25.6)	1.31 (0.55-3.12)	1.41 (0.56-3.55)
Yes	53 (68.8)	24 (31.2)		
Under ART years‡				
< 2	50 (70.4)	21 (29.6)	1.00 (0.44-2.28)	1.15 (0.45-2.90)
≥ 2	31 (70.5)	13 (29.5)		
Number of children				
≥ 2	74 (75.5)	24 (24.5)	3.85 (1.37-10.88)*	3.83 (1.30-11.30)
< 2	8 (44.4)	10 (55.6)		

* $p < 0.05$; † Total number of women who got pregnant after knowing of seropositive status was 132, but we excluded 16 participants who did not answer; ‡ 1 participant was excluded because she could not remember the answer; # Adjusted for age, religion, marital status, have paid job, formal education, under ART years, and number of children.

that understanding the prognosis of pediatric AIDS, and 'self-efficacy for asking condom use' were associated with their use of contraceptives.

4.1. *Unintended pregnancy*

We found a positive association between unintended pregnancies and having two or more children. In this study, we categorized pregnancies as "unintended" when women did not use contraceptives without an intention to have children or could not use contraceptives although she wanted to use them. WHO revealed that there were high unintended pregnancies among Rwandans reporting that an "unmet need of family planning" led to 37.9% of these unintended pregnancies in 2000-2006 in Rwanda, which is the second highest rate in the world, following 39.5% in the Lao People's Democratic Republic (*4*). Our study demonstrated that the unintended pregnancy rate of HIV positive women was also high, and was even higher than the national average. One of the hypotheses we can suppose from the results is that an HIV positive woman had to accept non-use of contraceptives due to their poor circumstances. As our result showed, 97.6% of women who got pregnant after knowing their seropositive status did not have paid jobs due to difficulty of finding work or/and keeping a job, and found it hard to live without a partner's support. According to the in-depth interviews of HIV positive women, which were conducted separately from this study by the first author, a considerable number of women responded that because they needed to rely on their partner to make a living for their family, they had to accept their partner's request for sexual behavior without using contraceptives, thus resulting in unintended pregnancy (*20*). If the number of children increased, then the more they needed their partner's financial support. These results may indicate the importance of economic independence of HIV positive women from their partners to avoid unintended pregnancies.

4.2. *Contraceptive use*

As for contraceptive use, it was positively associated with 'not agree that HIV positive children can survive as long as HIV negative children'. This result suggests that those who understood the prognosis of pediatric AIDS were more likely to use contraceptives. In other words, a correct understanding about what will happen to HIV positive children seems to restrain HIV positive women from getting pregnant, and as a result, it might reduce the number of pediatric AIDS cases. A previous study showed that concern over a child's future care could be a deterrent factor for pregnancy (*14*). However, the association between an understanding of the prognosis of pediatric AIDS and the non-use of contraceptives which our study revealed has not been shown in previous studies. Having a correct understanding of the prognosis of pediatric AIDS should be emphasized in the family planning counseling of HIV positive women. At the same time, it requires further investigation into what makes women believe that HIV positive children can survive as long as HIV negative children even though many HIV positive women are faced with their

Table 5. Factors associated with non-use of contraceptives among women who had sexual intercourse within three months (*n* = 263)

Variables	Use of contraceptives within three months		Crude OR (95% CI)	Adjusted OR[#] (95% CI)
	Yes (*n* = 178) (*n* (%))	No (*n* = 85) (*n* (%))		
Socio-economic variables				
Age (years old)				
≥ 35	74 (75.5)	24 (24.5)	1.81 (1.04-3.16)	1.42 (0.71-2.82)
< 35	104 (63.0)	61 (37.0)		
Religion				
Protestant	94 (67.6)	45 (32.4)	1.76 (0.83-3.74)	1.22 (0.46-3.23)
Catholic	56 (71.8)	22 (28.2)	0.46 (0.10-2.24)	0.60 (0.10-3.42)
Muslim	9 (81.8)	2 (18.2)	0.82 (0.45-1.51)	0.73 (0.34-1.54)
Other	19 (54.3)	16 (45.7)		
Marital Status				
Widowed/separated/divorced	41 (73.2)	15 (26.8)	1.51 (0.50-4.54)	0.67 (0.15-3.01)
Married/cohabiting	129 (66.8)	64 (33.2)	0.74 (0.38-1.43)	0.60 (0.26-1.39)
Single	8 (57.1)	6 (42.9)		
Have paid job				
Yes	15 (60.0)	10 (40.0)	0.69 (0.30-1.61)	1.28 (0.41-3.97)
No	163 (68.5)	75 (31.5)		
Formal education of women				
No	62 (67.4)	30 (32.6)	0.98 (0.57-1.68)	0.99 (0.49-2.00)
Yes	116 (67.8)	55 (32.2)		
Under ART years†				
≥ 2	83 (66.9)	41 (33.1)	1.02 (0.61-1.72)	0.86 (0.44-1.69)
< 2	91 (67.4)	44 (32.6)		
Pregnancy related variables				
Number of children				
≥ 2	121 (67.2)	59 (32.8)	0.94 (0.54-1.64)	0.74 (0.32-1.72)
< 2	57 (68.7)	26 (31.3)		
Ever experienced the death of a child				
Yes	93 (73.2)	34 (26.8)	1.64 (0.97-2.77)	1.61 (0.82-3.16)
No	85 (62.5)	51 (37.5)		
Counseling experiences after knowing sero-positive status on				
How to discuss with partner about family planning				
No	63 (61.2)	40 (38.8)	1.62 (0.96-2.74)*	1.28 (0.67-2.43)
Yes	115 (71.9)	45 (28.1)		
Desire to have children				
No	149 (68.7)	68 (31.3)	1.28 (0.66-2.50)	1.77 (0.65-4.79)
Yes	29 (63.0)	17 (37.0)		
HIV knowledge related variables				
All correct	98 (68.5)	45 (31.5)	1.09 (0.65-1.83)	1.27 (0.67-2.43)
Not all correct	80 (66.7)	40 (33.3)		
Perception related variables				
Think HIV positive babies can survive as long as HIV negative babies‡				
No	104 (73.2)	38 (26.8)	1.72 (1.00-2.93)*	2.28 (1.12-4.63)
Yes†	67 (61.5)	42 (38.5)		
Self-efficacy variables				
Can ask their partner to use condom				
Always	125 (86.8)	19 (13.2)	6.39 (3.25-12.55)*	9.83 (4.28-22.61)
Sometimes	18 (36.0)	32 (64.0)	11.70 (5.51-24.82)*	11.12 (4.89-25.29)
Never	35 (50.7)	34 (49.3)		

* $p < 0.05$; † 4 participants were excluded because they could not remember the answer; ‡ 12 participants were excluded because they did not respond to this question; [#] Adjusted for age, religion, marital status, have paid job, formal education, under ART years, number of children, experienced death of children, counseling experience, desire to have children, MTCT knowledge, 'HIV positive babies can survive as long as HIV negative babies', and can ask their partner to use a condom.

child's death. In our study, almost half of the women who had ever gotten pregnant had experienced a child's death (48.3%).

Furthermore, we found a strong negative association between the non-use of contraceptives and the level of high self-efficacy, that they can always/sometimes ask their partner to use condoms. Though the number of applicable respondents for this question was low, a high significance shows the importance of self-efficacy for contraceptive use. Previous studies also suggested that encouraging women to raise their self-efficacy might be positively related to their contraceptive use (*21*,*22*). Initiation of the practical skills for condom negotiation should be included in the family planning counseling of

HIV positive women.

In contrast, the desire to have children was not found to be a predictor of the non-use of contraceptives, although a study in Uganda showed a significant association between them (*10*). Our results might indicate that women do not use contraceptives, regardless of whether they desire to have children or not. In other words, it implies that there exists a high number of unintended pregnancies. In fact this study showed a very high unintended pregnancy rate (62.7%) among those who had gotten pregnant after knowing their seropositive status.

This study has some limitations. First, traditional contraceptive methods such as the extension of breast-feeding which might restrain ovulation and the utilization of the safety period were not included. We examined only the cases of modern contraceptive methods which need the user's strong willingness to prevent pregnancy for utilization. Therefore, in our study, contraceptive users reflected that they had real intentions to prevent pregnancy. Second, the possibility of recall bias should be considered to measure whether women had the intention of pregnancy at the moment of sexual behavior when they got pregnant. However, getting pregnant is such a significant event that women usually can remember whether they had the intention of getting pregnant or not. Our results, thus, might not be strongly affected by the possibility of this limitation. Finally, our results might not be generalized to all HIV positive women because we selected our participants using a convenient sampling method. However, most of the women in our study sites had appointments at monthly intervals. With this, we assume that most of the women who met our criteria were included in our study.

In conclusion, this study suggests that HIV positive women under ART need special support to avoid unintended pregnancies especially those who have two or more children. Moreover, interventions are also needed to improve an understanding of the prognosis of pediatric AIDS and condom-use negotiation skills among HIV positive women, because these factors were associated with the non-use of contraceptives.

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Depression and health-related quality of life after discharge and associated factors in childhood cancer patients in Japan

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Summary **We identified the prevalence of depression and quality of life (QOL) of Japanese children with childhood cancer after discharge using the Birleson Depression Self-rating Scale for Children (DSRS-C) and the Pediatric Quality of Life Inventory (PedsQL). Subjects were 118 caregivers who raised children ages 2-18 with childhood cancer; subjects resided in suburban districts of Japan and completed instruments after their children were discharged. Multiple regression analysis of data collected from 105 respondents revealed that lower PedsQL scores correlated with more problems in life at school and at home, an increased frequency of hospital visits, less cooperation within the family, and higher DSRS-C scores. To ensure the QOL of children with childhood cancer, outpatient nurses need to encourage children to psychosocially adapt after discharge, periodically screen children during outpatient treatment using instruments such as the DSRS-C, and conduct preventive interventions for children who meet screening criteria and their families before they suffer from adaptation disorders and offer multilateral psychosocial assistance in cooperation with a multidisciplinary care team.**

Keywords: Childhood cancer, health-related quality of life (HRQOL), depression, family cohesion, Japan

1. Introduction

Childhood cancer is a generic term for malignant neoplasms that occur in childhood, with hematopoietic malignant tumors such as leukemia and malignant lymphoma accounting for approximately half of childhood cancers, followed by other solid tumors, such as a brain tumor, neuroblastoma, Wilms' tumor, and rhabdomyosarcoma (*1*). The recent development of new treatment strategies has greatly improved clinical outcomes, with a 5-year disease-free survival rate of approximately 80% for patients with acute lymphoid leukemia (ALL), 50% for those with acute myeloid leukemia (AML), 85-100% for those with malignant lymphoma (non-Hodgkin's disease), 92-100% for those with neuroblastoma (Stages 1 and 2), 80-96% for those with Wilms' tumor, and 75% for those with rhabdomyosarcoma; this survival rate differs, however, depending on the stage and the status of metastasis and recurrence of disease (*2*). The number of individuals who have experienced childhood cancer has increased in recent years.

The treatment of childhood cancer requires long-term hospitalization and imposes physical and psychological burdens on children with childhood cancer (*3*). The treatment of leukemia involves four stages: *i*) induction therapy to achieve a response (a reduction in leukemia cells to less than 5% in the bone marrow), *ii*) intensification therapy, *iii*) CNS

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prophylaxis, and *iv*) maintenance therapy. Generally, childhood cancer patients in Japan require nearly one year of hospitalization and two to three years of outpatient treatment (*2*). In the event of a solid tumor, surgical excision of the primary tumor is frequently performed before the chemotherapy mentioned earlier and is often combined with radiotherapy. A lumbar puncture and bone marrow aspiration performed at each stage of treatment and intense side effects of therapeutic agents (*e.g.* nausea, fatigue, and headaches) place a physical burden on the children. Changes in body image such as hair and weight loss and living apart from one's family and friends impose a psychological burden on the children. The families of such children also have physical burdens (*e.g.* going back and forth between home and the hospital, housekeeping, caring for siblings, nursing of the ill child in the hospital) and psychological burdens (the child's fear of having cancer and caring about other family members, especially siblings) and fatigue (*4,5*).

Many children who have completed painful anti-cancer treatment may suffer direct or indirect damage (late effects), such as treatment-related damage to the cranial nerves and the heart, functional deficits following tumor resection, post-transfusion hepatitis, and failure to grow; these children may also have various psychosocial problems (*6-8*). As a result, various studies have been conducted regarding the quality of life (QOL) and psychosocial adaptation of childhood cancer survivors (*9*).

In Japan, investigations of the QOL of childhood cancer survivors/children have just started. A Japanese version of the Pediatric Quality of Life Inventory (PedsQL) was developed in 2010; PedsQL assesses health-related QOL (HRQOL), and the reliability and validity of the Japanese version have been tested (*10*). The background for the treatment of childhood cancer and the cultural background of the parent-child relationship/family relationship in Japan differ from those in other countries. In Japan, for example, a child is often informed of his or her illness, but many parents still hesitate to inform children of the fact that they are ill (*11*). Children are often poorly and improperly informed of their illness. In fact, children are informed of their illness at the age of 15, on average, and in many instances younger children are not informed of the fact that they have cancer. Previous studies conducted in Japan on the same topic have reported on the mental health, anxiety, and adaptive process of children after discharge (*12,13*). The reality of outcomes has been determined, but relevant factors that will guide interventions and assistance have not been analyzed.

This study looked at family cohesion (the degree of cooperation and family functioning) during hospitalization and after discharge from the viewpoint of "a child living with his or her family". This study sought to assess the prevalence of depression and HRQOL of the children and associated factors by focusing on the psychosocial adaptation of children with childhood cancer after discharge.

2. Methods

2.1. *Subjects*

Subjects were children who made periodic visits to the Pediatric Department of A University Hospital and the Hematology and Oncology Department of B Pediatric Hospital and their primary family caregivers. The inclusion criteria were: *i*) being a child from primary school age to age 18 who had been hospitalized for childhood cancer or being a family caregiver who primarily provided care to a child over 2 years of age during the child's hospitalization for cancer and *ii*) being able to complete a physical and psychological questionnaire (in about 20 minutes) administered by the child's primary physician.

2.2. *Data collection*

Potential subjects were recruited by primary physicians using the inclusion criteria. Subjects who consented to participate in the study were provided with detailed information about the study and asked to complete a questionnaire. The subjects could answer the questionnaire either at the hospital while waiting for consultation or payment or at home. Subjects who chose to fill in the questionnaire at the hospital were asked to submit it to the Outpatient Department, and subjects who completed the questionnaire at home were asked to return it by mail using the nearest post office.

The questionnaire included items on *i*) the characteristics of the children and their families, *ii*) the prevalence of depression and the QOL of the children after discharge, and *iii*) possible factors related to *ii*).

Subject characteristics of children and their families were determined by asking family caregivers about their child's current age and about "the number of family members" and "siblings".

The prevalence of depression and the QOL of the children were determined by administering the Japanese version of the Birleson Depression Self-rating Scale for Children (DSRS-C) to the children and the Japanese version of the Pediatric Quality of Life Inventory™ (PedsQL™) to the family caregivers. The DSRS-C is an 18-item self-rating scale that was developed by Birleson to screen for the presence of depression in children (*14*) and was translated in Japanese by Murata *et al*. (*15*) (*ex.* 'I sleep very well' – item 2; 'I feel like crying' – item3; 'I have horrible dreams' – item 14; 'I feel very lonely' – item 15). The children are asked to rate their own state during the last week on a 3-point scale. The answers are scored from 0 to 2 with a total score of 36 points. The cut-off score is set at 16 points.

The PedsQL is a 23-item measure that was designed for parents by Varni *et al*. (*16*) to evaluate the HRQOL of children (*ex.* 'lifting something heavy' – item 4; 'feeling sad or blue' – item 11; 'getting along with other children' – item 14; 'missing school because of not feeling well' – item 22). The Japanese version has been tested for reliability and validity by Kobayashi & Kamibeppu (*10*). The current study used the overall score on the PedsQL, which includes the following sub-constructs: physical, emotional, social, and school functioning. The higher the total score, the better the HRQOL.

Family caregivers were asked about their child's "age of onset", "illness (type of cancer)", "type of treatment given", "status of recurrence", "frequency of hospital visits", "whether or not [the] child has been informed of his or her illness", the "frequency of visits by family caregivers", the "sense of burden felt by family caregivers while providing care", the "degree of cooperation within the family", "support from outside the family" during the child's hospital stay, and "sequelae in [the] child". Caregivers were also asked "whether or not [the] child will be able to return to school", "problems encountered by [the] child in daily life", "limitations imposed on [the] child's daily life", "whether or not [the] child has been informed of his or her illness", the "sense of burden felt by family caregivers while providing care", the "degree of cooperation within the family", and "support from outside the family" after discharge. The sense of burden and the degree of cooperation were assessed using a Likert five-point scale. Furthermore, the caregivers were asked to complete the FACES SKGIV to assess family functioning (*17*).

The children were asked about "the hardest and most distressing time and event that [they] experienced while ill", and their "bounce back" to the present was assessed on a six-point face scale. Data were collected from June 2010 to March 2011.

2.3. *Data analysis*

Questionnaires with more than 90% of the questions completed were defined as valid responses and were subjected to analysis.

Descriptive statistics were determined by calculating distributions, means, and standard deviations of responses for each variable regarding subject characteristics, the child's depression and QOL, and possible factors associated with the child's QOL. Next, univariate analysis of PedsQL scores and each possible associated factor was performed prior to multivariate analysis for factors associated with each outcome. Spearman rank-correlation coefficients were used for continuous variables, a *t*-test for dichotomous variables, and analysis of variance for polychotomous variables. If no multicollinearity was found among related factors, then multiple regression analysis was performed with PedsQL scores as objective variables and possible associated factors as explanatory variables.

A statistical analysis package, PASW Statistics 18.0 for Windows (SPSS Japan Inc.), was used for analysis. The significance level was set at 5%.

2.4. *Ethical considerations*

Potential subjects were informed verbally and in writing that: *i*) the subject's cooperation with the study was entirely voluntary, *ii*) a child would not be penalized in terms of medical care or treatment if the caregiver did not cooperate with the study, *iii*) consent to participate in the study could be withdrawn at any time during the study; and *iv*) when the study results were published the privacy of subjects would be strictly protected.

The study was approved by the medical ethics review board of the University of Tsukuba and Ibaraki Children's Hospital.

3. Results

3.1. *Summary of subject characteristics*

Of 118 family caregivers who consented to participate in the study, 105 completed more than 90% of the questionnaire. Of 100 children with childhood cancer, 90 completed more than 90% of the questionnaire; the remaining children were preschool age or severely ill.

Table 1 shows the characteristics of children and their caregivers and families. The most common "illness (type of cancer)" was acute lymphoblastic leukemia (40 patients), followed by acute myeloid leukemia (12 patients) and neuroblastoma (7 patients). "The age of onset" was 5.5 ± 3.7 (mean ± S.D.) years and the "current age" was 11.5 ± 8.2 (mean ± S.D.) years. The "length of hospital stay" was 11.2 ± 6.9 (mean ± S.D.) months, and 15 patients experienced "recurrence" and 3 had repeated recurrence. All of the children received chemotherapy, 39 underwent surgery, 33 underwent radiotherapy, and 17 underwent transplantation. After admission, the children lived with 3.7 ± 1.4 (mean ± S.D.) family members and had 1.2 ± 0.9 (mean ± S.D.) siblings.

3.2. *Current status of depression and HRQOL of children with childhood cancer*

Table 2 shows the mean scores, S.D., and the range of DSRS-C and PedsQL scores.

3.3. *Factors associated with the HRQOL of children with childhood cancer*

The original plan was to conduct two sets of multiple regression analyses using the DSRS-C scores and the PedsQL scores shown in Table 2 as objective variables,

Table 1. Subject Characteristics (*n* = 105)

Variables	*n*/mean ± S.D.	%/range
Children		
Current age	11.5 ± 8.2	2-18
Age of onset	5.5 ± 3.7	0-17
Illness (type of cancer) [multiple answers permitted]		
ALL	40	38.1%
Ph+ALL	5	4.8%
ALL with Down syndrome	1	1.0%
AML	12	11.4%
Neuroblastoma	7	6.7%
Rhabdomyosarcoma	6	5.7%
ML	6	5.7%
LCH	4	3.8%
Hodgkin's lymphoma	3	2.9%
Medulloblastoma	3	2.9%
APL	2	1.9%
AL	2	1.9%
Non-Hodgkin's lymphoma	2	1.9%
Burkitt lymphoma	2	1.9%
Embryoma	2	1.9%
Germ cell tumor	2	1.9%
Wilms' tumor	1	1.0%
T-cell lymphoma	1	1.0%
Retinoblastoma	1	1.0%
Rrimitive neuroectodermal tumor	1	1.0%
Pineoblastoma	1	1.0%
Osteoblastoma	1	1.0%
Lymphocytic leukemia	1	1.0%
Germinoma	1	1.0%
Brain tumor	1	1.0%
Type of treatment given		
Chemotherapy	105	100.0%
Radiotherapy	33	31.4%
Surgery	39	37.1%
Transplantation	17	16.2%
Other	2	1.9%
Status of recurrence		
Yes	15	14.3%
No	90	85.7%
Frequency of hospital visits per year	9.1 ± 7.3	1-48
Return to school		
Yes	92	87.6%
No	13	12.4%
Sequelae		
Yes	37	35.2%
No	68	64.8%
Problems in daily life		
Currently	33	31.4%
Temporarily in the past	44	41.9%
No	26	24.8%
Limitations on daily life		
Yes	15	14.3%
No	90	85.7%
Bounce back from the most distressing time (*1-6*)	5.1 ± 1.0	2-6
Family caregivers		
During hospitalization		
Frequency of visits	17	16.2%
Stayed for 24 hours	28	26.7%
All the time while the child was awake	30	28.6%
Almost the entire time during the day	29	27.6%
A few hours during the day		
Was the child informed of his/her illness		
Yes	47	44.8%
Somewhat (but not that he/she had cancer)	35	33.3%
No	21	20.0%

To be continued

Continued

Variables	*n*/mean ± S.D.	%/range
Sense of burden while providing care		
Yes, very much so	18	17.1%
Yes	33	31.4%
Somewhat	39	37.1%
No	13	12.4%
No, none at all	1	1.0%
Cooperation within the family		
Frequent	66	62.9%
Sometimes	26	24.8%
On occasion	8	7.6%
Not much	3	2.9%
None at all	2	1.9%
Support from outside the family		
Frequent	58	55.2%
Sometimes	20	19.0%
On occasion	10	9.5%
Not much	13	12.4%
None at all	4	3.8%
After discharge to present		
Was the child informed of his/her illness		
Yes	61	58.1%
Somewhat (but not that he/she had cancer)	19	18.1%
No	21	20.0%
Sense of burden while providing care		
Yes, very much so	2	1.9%
Yes	8	7.6%
Somewhat	26	24.8%
No	39	37.1%
No, none at all	30	28.6%
Cooperation within the family		
Frequent	39	37.1%
Sometimes	44	41.9%
On occasion	18	17.1%
Not much	3	2.9%
None at all	1	1.0%
Support from outside the family		
Frequent	40	38.1%
Sometimes	19	18.1%
On occasion	20	19.0%
Not much	17	16.2%
None at all	8	7.6%
Family members		
Number of family members living with the child	3.7 ± 1.4	1-7
Number of siblings	1.2 ± 0.9	0-4
FACES-cohesion		
Enmeshed	55	52.4%
Connected	40	38.1%
Separated	10	9.5%
Disengaged	0	0.0%
FACES-adaptability		
Rigid	8	7.6%
Structured	75	71.4%
Flexible	18	17.1%
Chaotic	4	3.8%

Abbreviations: ALL, acute lymphatic leukemia; AML, acute myelogenous leukemia; ML, malignant lymphoma; LCH, Langerhans cell histiocytosis; APL, acute promyelocytic leukemia; AL, acute leukemia.

but multiple regression analysis was performed once using the scores of PedsQL as objective variables and subject characteristics and other possible relevant factors as explanatory variables since there was a significant negative correlation between PedsQL scores and DSRS-C scores ($r = -0.29$, $p < 0.01$).

Table 3 shows factors associated with the HRQOL of children with childhood cancer. More problems in life at school and at home (standard partial regression coefficient [sb] = −0.298, $p = 0.009$), an increased frequency of hospital visits (sb = −0.281, $p = 0.017$), less cooperation within the family (sb = 0.247, $p = 0.034$), and higher DSRS-C scores ($\beta = -0.221$, $p = 0.025$) were correlated with lower PedsQL scores (F =

Table 2. Scores on the Japanese versions of PedsQL™ and DSRS-C

	No. of items	Score range	Mean ± S.D.	Range
Scores on the Japanese version of PedsQL™	34	0-100	79.32 ± 18.14	(*n* = 105) 38.1-100
Scores on the Japanese version of DSRS-C	18	0-36	6.98 ± 4.17	(*n* = 90) 0-18

PedsQL™: Higher scores indicate better HRQOL; DSRS-C: Higher scores indicate worse depression.

Table 3. Factors related to the HRQOL[1)] of childhood cancer survivors (*n* = 105)

Factors	sb	*p*-value
Children		
Current age	0.11	0.28
Age of onset	−0.01	0.96
Illness (type of cancer)	--	--
Type of treatment given (Radiotherapy/surgery/transplantation/other[#])	0.12	0.31
Status of recurrence (Yes/No[#])	0.05	0.65
Frequency of hospital visits per year	−0.28	0.02*
Return to school (Yes/No[#])	0.07	0.50
Sequelae (Yes/No[#])	−0.01	0.92
Problems in daily life (1: No, none at all, 2: Sometimes, 3: Consistently)	−0.3	< 0.01**
Limitations on daily life (Yes/No[#])	0.01	0.93
Bounce back from the most distressing time (*1-6*)	−0.14	0.23
Status of depression[2)]	−0.22	0.03*
Family caregivers		
During hospitalization		
Frequency of visits (1: A few hours during the day, 2: Almost the entire time during the day, 3: All the time while the child was awake, 4: Stayed for 24 hours)	−0.06	0.58
Was the child informed of his/her illness (1: No, 2: Somewhat, 3: Yes)	0.02	0.84
Sense of burden while providing care (1: No, none at all, 2: No, 3: Somewhat, 4: Yes, 5: Yes, very much so)	−0.09	0.41
Cooperation within the family (1: None at all, 2: Not much, 3: On occasion, 4: Sometimes, 5: Frequent)	0.11	0.35
Support from outside the family (1: None at all, 2: Not much, 3: On occasion, 4: Sometimes, 5: Frequent)	−0.08	0.42
After discharge to present		
Was the child informed of his/her illness (1: No, 2: Somewhat, 3: Yes)	0.05	0.63
Sense of burden while providing care (1: No, none at all, 2: No, 3: Somewhat, 4: Yes, 5: Yes, very much so)	−0.13	0.25
Cooperation within the family (1: None at all, 2: Not much, 3: On occasion, 4: Sometimes, 5: Frequent)	0.25	0.03*
Support from outside the family (1: None at all, 2: Not much, 3: On occasion, 4: Sometimes, 5: Frequent)	−0.1	0.35
Family members		
Number of family members living with the child	−0.06	0.58
Number of siblings	0.03	0.78
Cohesion (1. Disengaged, 2. Separated, 3. Connected, 4. Enmeshed)	−0.11	0.30
Adaptability (1. Chaotic, 2. Flexible, 3. Structured, 4. Rigid)	0.008	0.94
R^2	0.4	
Adjusted R^2	0.31	

sb: the values are standardized partial regression coefficients; * $p < 0.05$, ** $p < 0.01$; [#] reference category; -- Variables that were not selected as model variables as a result of variable selection; [1)] Assessed using the Japanese version of PedsQL™; [2)] Assessed using the Japanese version of DSRS-C.

4.155, $p < 0.001$).

The problems frequently encountered by the children in their daily life included feeling fatigued and a sense of isolation because of "feeling weak", loss of confidence and difficulty with social interaction due to "changes in body image", and underachievement, feeling lonely, or feeling anxious due to "inconsistent school attendance when being treated to prevent infection". In some instances, "underachievement" and "feeling left out by friends" discouraged the

child's school attendance. Some children had physical complaints caused by social stress, such as headaches or abdominal pain, or psychological instability, such as a temper and melancholy, at home.

4. Discussion

This is the first study in Japan to assess the prevalence of depression and HRQOL of children and associated factors from the viewpoint of "a child living with his or her family". Results of this cross-sectional questionnaire-based study offer suggestions for the support needed by caregivers and families raising children with childhood cancer.

4.1. *Actual depression and HRQOL of children with childhood cancer*

The current subjects had DSRS-C scores, which indicate one's level of depression, that were comparable to those reported in previous studies [8.79 ± 4.57 (mean ± S.D.) as reported by Uchida & Fujimori (*18*) and 12.55 ± 6.42 (mean ± S.D.) as reported by Nagai (*19*)]. Only 3 subjects had scores above the cut-off. Based on these results, factors including cancer morbidity and treatment may affect depressive tendencies in individual children, but they had little effect on subjects as a whole in this study.

A previous study found that the Japanese version of the PedsQL was applicable to community and school health settings and useful in clinical settings (*10*). That study involved healthy children, including those with chronic health needs and mental conditions. In that study, toddlers and young children had a total PedsQL score of 83.1 ± 13.8 (mean ± S.D.) (range: 33.3-100), school children and adolescents had one of 84.1 ± 13.3 (mean ± S.D.) (range: 42.4-100), and subjects overall had one of 83.91 ± 13.4 (mean ± S.D.) (range: 33-100). These scores are higher than the PedsQL score of 79.3 ± 18.1 (mean ± S.D.) (range: 38.1-100) for children ages 2 to 18 with childhood cancer in the current study. Hao *et al.* also noted that healthy children reported higher scores than pediatric leukemia patients ($p < 0.001$) although the children were in a different country; healthy children had a total score of PedsQL of 82.38 ± 13.29 (mean ± S.D.) while children with leukemia had one of 56.72 ± 20.35 (mean ± S.D.) (*20*). Whether children with cancer have high or low scores will depend on the differences in medical care and cultural background of the country in question. In either instance, the factors of cancer morbidity and treatment may have a major impact on the HRQOL of children.

4.2. *Factors associated with the HRQOL of children with childhood cancer*

Results of multivariate analysis suggested that having problems in daily life, an increased frequency of hospital visits, less cooperation within the family after the child's discharge, and an increased tendency for the child to experience depression were correlated with a low HRQOL for the child.

This study showed that children with childhood cancer encountered problems in daily life after discharge, including feeling fatigued and a sense of isolation because of "feeling weak", loss of confidence and difficulty with social interaction due to "changes in body image", and underachievement, feeling lonely, or feeling anxious due to "inconsistent school attendance when being treated to prevent infection". Suzuki *et al.* also reported four categories of characteristic problems encountered by Japanese children with childhood cancer after discharge: "physical difficulties", "behavioral difficulties", "interpersonal difficulties" and "fear of the future"; they also suggested that physical and behavioral problems may develop into social problems such as problems with interpersonal relationships and view of the future over time (*21*). In cooperation with a multidisciplinary care team consisting of physicians, social workers, psychological specialists, school teachers, and other members, nurses are expected to provide long-term support that takes into account both the status of outpatient treatment and the life stage of their patients.

Meeting and talking with the attending physician during hospital visits can reduce the fear felt by parents. A study of families of children with chronic diseases reported that an increased frequency of patients' hospital visits is associated with greater family empowerment. In the current study of children with childhood cancer, an increased frequency of hospital visits indicated that "little time had passed since treatment started", "[the] child's condition was not stable", or "a high risk of recurrence". That is, children with childhood cancer who have an increased health risk and require professional supervision will visit the hospital more frequently. Redaelli *et al*. noted a positive correlation between the HRQOL of children with childhood cancer after discharge and the "time since treatment" (*22*), but the current study is the first to demonstrate a significant correlation between the HRQOL after admission and the frequency of hospital visits. Maurice-Stam *et al.* noted that a better HRQOL was associated particularly with more positive expectations of the further course of the disease and less frequent parental asking after disease-related emotions of the child (*23*). Children who visit the hospital more frequently are presumed to have more difficulty being hopeful about the future and such children and their parents are both presumed to be more prone to fear of disease because of their health risks. Thus, children visiting the hospital more frequently need more psychosocial support from a multidisciplinary care team of childhood cancer specialists.

Data on the degree of cooperation within the family were collected during hospitalization and after discharge. The HRQOL of the child was found to be significantly correlated with the degree of cooperation within the family "after discharge". The families of children with childhood cancer have more things to do on their own after discharge than during hospitalization. The family is responsible for escorting the child to the hospital for a couple of years, managing medication, coordinating school attendance (while monitoring of the potential for infection), solving various problems at school, and careful care of the child. If all of these responsibilities are borne by one family member (*e.g.* the mother), that family member will be under considerable psychological pressure and feel fatigued. Previous studies noted that the anxiety and emotional fluctuations experienced by parents will be transmitted to their children (*24*,*25*). The HRQOL of the child may be negatively affected. Outpatient nurses are expected communicate with parents accompanying their children to the hospital in order to assess the child's family life, including the system of family cooperation and, if necessary, share information with medical social workers, local public health nurses, and school nurses and intervene in the family.

The negative correlation between the level of depression (DSRS-C scores) and HRQOL (total PedsQL score) has also been described by Kobayashi & Kamibeppu (*10*). Maurice-Stam *et al.* have indicated that many childhood cancer survivors who have remained in remission may have several psychosocial problems in addition to treatment-related physical problems (*3*). The problems experienced by the children include depression, emotional disturbance, PTSD, and withdrawal. Family issues include prolonged parental anxiety, PTSD, and maladjustment of siblings. In the present study, some children had adaptation disorders, *e.g.* loss of voice after being bullied by friends, and fell behind in schoolwork or had difficulty developing friendships due to frequent absences from school because of the fear of infection. These issues led to inconsistent school attendance. However, overall levels of depression (DSRS-C scores) were not high as those reported in previous studies. Schultz *et al.* noted that "having cancer of the central nervous system" and "undergoing cancer therapy involving the central nervous system" (such as brain radiation or chemotherapy injected into the spinal fluid) are factors that contribute to depression in children (*26*). However, the current study found no significant differences in levels of depression (DSRS-C scores) for patients with "cancer of the central nervous system" and patients with another form of cancer. Since high levels of depression are associated with a low HRQOL in children with cancer after their discharge, results suggested that outpatient staff members need to prevent and address both depression and a low HRQOL in children with childhood cancer. In particular, outpatient nurses should: *i*) periodically screen children during outpatient treatment using instruments such as the DSRS-C and *ii*) conduct preventive interventions for children who meet screening criteria and their families before they suffer from adaptation disorders and offer multilateral psychosocial assistance in cooperation with a multidisciplinary care team of childhood cancer specialists.

4.3. *Study limitations or directions for future research*

This study advanced the hypothesis that family functioning may influence HRQOL or depression of children with childhood cancer, but the hypothesis was not tested because most families rearing children with childhood cancer had stylized family functioning, *i.e.*, "enmeshed cohesion" and "structured adaptability". In future research, the authors wish to focus on a stylized family functioning and parental care of children who have cancer after discharge and discuss the role of outpatient nurses and the multidisciplinary care team.

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Case Report

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Sporadic case infected by severe fever with thrombocytopenia syndrome bunyavirus in a non-epidemic region of China

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Summary **We report here a clinical and molecular study on a case suffer from severe fever with thrombocytopenia syndrome (SFTS) due to a new type of bunyavirus, named SFTS bunyavirus (SFTSV), in Zhejiang Province China. The key clinical features of this patient include fever, lymphocytopenia and thrombocytopenia. We carried out a serological and molecular investigation in the indicated case and on relatives with close contact. The SFTSV infection was confirmed through amplification of viral genetic material using the polymerase chain reaction (PCR) from the patient's serum, but not relatives with close contact. Subsequently direct sequence of PCR product demonstrated a homology of 94-96% in the nucleotide sequence compared to a reference sequence previously reported, in which the majority of patients originated from an epidemic area of Central and Northeast China. Our results suggest that SFTSV can occur in a non-epidemic area due to a similar strain of SFTSV that apparently affect the blood system, implying the importance of dissecting the pathogenesis of SFTS as well as mode of infection.**

Keywords: Severe fever with thrombocytopenia syndrome bunyavirus (SFTSV), molecular diagnosis, sporadic infection

1. Introduction

Severe fever with thrombocytopenia syndrome bunyavirus (SFTSV) infection, a type of RNA virus and a new member of Bunyaviridae belonging to *phlebovirus*, was first identified by the Chinese Center for Disease Control and Prevention in 2010 (*1*). severe fever with thrombocytopenia syndrome (SFTS) mainly occurs in spring and summer, with middle-aged residents in hilly regions most susceptible. The major manifestations of SFTSV infection include fever, thrombocytopenia, gastrointestinal symptoms, and leukocytopenia (*2*). However, the pathogenesis of SFTSV remains unclear and the epidemiological data are still limited. Up to September 2010, SFTS had only been reported in Central and Northeast China (*2*). We report here a patient with suspected SFTSV infection who was admitted to Zhoushan People's Hospital, Zhejiang Province of China in the middle of June 2011, and was finely diagnosed using the polymerase chain reaction (PCR) of the target genes of the RNA-dependent RNA polymerase, the glycoprotein and N protein in the conserved core region of novel bunyavirus, and nucleotide sequence analysis.

2. Case report

The patient was a 72-year-old female who had a

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sudden onset of fever (the highest temperature was 39.1°C), accompanied by chills and pharyngeal pain. After accepting intravenous ribavirin and clindamycin for one day without remission, she was referred to the local hospital with a preliminary diagnosis of fever and thrombocytopenia of undetermined origin. Thereafter she was treated with intravenous piperacillin/tazobactam, levofloxacin tablets, solu-medrol and frozen fresh plasma for a week, but no clinical improvement was observed. On the eighth day, since she developed melena and generalized myalgia, the patient was subsequently transferred to the emergency room of the general hospital located in Zhoushan city for further treatment. Upon arrival, she was conscious but listless and pale, with temperature of 37.5°C, and pulse rate of 98 beats/min Her respiratory rate was 19 times/min, and her blood pressure was 104/68 mmHg. There were no overt sign of jaundice, and no petechia or ecchymoses were noted. Enlarged lymph nodes were palpable on both sides of the neck, in the axillary and inguinal regions. Her posterior pharyngeal wall was congested, and her vesicular breath sounds were abnormal. Her abdomen was soft, without rebound tenderness, shifting dullness or percussion tenderness over the hepatic and renal regions and no hepatosplenomegaly. Her lower limbs were not edematous, and neurological signs were negative.

Hematology showed leukopenia (total count 2.6 × 10^9/L, lymphocytes 0.36 × 10^9/L and neutrophils 1.98 × 10^9/L) and thrombocytopenia (48 × 10^9/L). Her lymphocyte subsets count showed that $CD4^+$ and $CD8^+$ T cells were significantly decreased, especially the $CD4^+$ T cells (52.4 × 10^6/L). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated to 710 U/L and 129 U/L, respectively. Creatine kinase (CK) was 671 U/L, albumin 19.8 g/L, and lactate dehydrogenase (LDH) 3,669 IU/L. Urinalysis showed moderate proteinuria and hematuria. Fecal occult blood test was strongly positive, consistent with the melena. A non-contrast CT scan of the chest showed mild inflammation in the superior lobe of the right lung, as well as emphysema in both lungs with pleural thickening and adhesions indicative of chronic bronchitis. The non-contrast CT scan of the abdomen was normal (Figure 1).

Considering the high probability of active gastrointestinal bleeding, intravenous cefmetazole and omeprazole sodium for injection were prescribed, along with reduced glutathione tablets. No improvement was seen after three days' treatment. The patient's platelet count decreased further to 12 × 10^9/L on the 11th day. She also experienced mild renal insufficiency, hypokalemia and hyponatremia. Her thrombin time was mildly abnormal and fibrinogen degradation products were slightly elevated. All of the signs and symptoms suggested that the patient was suffering from SFTS. Meanwhile, the patient was prescribed bed rest and was given a semi-liquid diet, along with symptomatic and supportive therapy as well as close monitoring of vital signs and urine output. Intravenous ribavirin and broad-spectrum antibiotics were prescribed for the empiric treatment of viral and bacterial infection. Since serum albumin levels and platelet count were severely depressed, and symptoms of gastrointestinal bleeding persisted, nutritious supportive therapy was also used. In addition, human immunoglobulin was injected intravenously to enhance the immunity of the patient. The treatments were effective and the patient was discharged after 20 days.

The patient had worked as a farmer for more than fifty years in hilly regions of Zhoushan Island, Zhejiang province, and had not travelled to other places for many years. She was not rearing poultry. She had worked in the cornfield two weeks before the onset of fever, but had no history of tick bites or contacts with individuals who had similar manifestations. There were rodents living in the areas she inhabited, and poor sanitary condition in her domicile. No other individuals in her village or neighboring villages presented with similar symptoms.

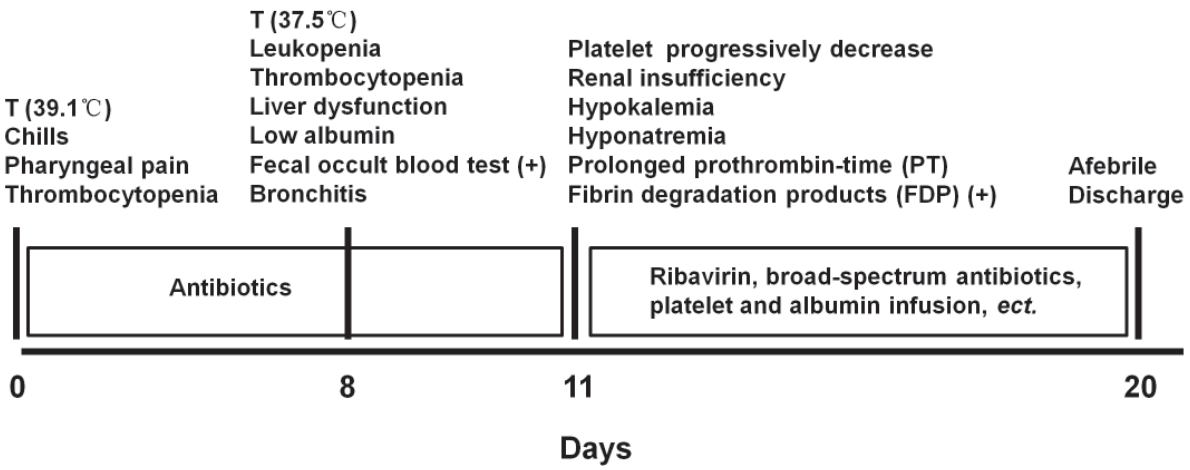

Figure 1. Summary of clinical findings.

Table 1. Sequences of primers

Direction	Sequence (5'-3')
bun rdrp-S	ATGGACAACCCTGCATTCG
bun rdrp-AS	TCAGCTTCTAGGCTAAAACCAG
bun gly 601-S	AAGAGTTTTAGCCAAAGTGAATTCCC
bun gly 1753-AS	ACATTCCTTCATATTTCCGCTCCC
bun NS 1043-S	CTTCAGCCACTTCACCCGAAC
bun NS 1646-AS	GCAGCAGCTCAATTTGACT

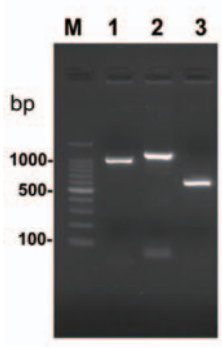

Figure 2. PCR amplification of RNA-dependent RNA polymerase, glycoprotein and N protein genes from blood DNA of a patient with severe fever and thrombocytopenia syndrome (SFTS). The amplified DNA fragment of RNA-dependent RNA polymerase with primer bun rdrp-S and bun rdrp-AS (**lane 1**). The amplified DNA fragment of glycoprotein with primer bun gly601-S and bun1753-AS (**lane 2**), and the amplified DNA fragment of N protein with primer bun NP 69-S and bun NP 606-AS (**lane 3**). **M**, DNA size marker (100 bp ladder).

The diagnosis of SFTSV infection was confirmed by DNA assay using detection of the target genes of the RNA-dependent RNA polymerase, the glycoprotein and N protein in the conserved core region of a novel bunyavirus by PCR. Viral RNA was extracted from the blood sample of the patient with a QIAampMinElute virus spin kit according to the manufacturer's instructions, and used to synthesize the single-strand cDNA. The single-strand cDNA was amplified by RT-PCR with random primers by using a ProtoScriptFirst Strand cDNA synthesis kit (New England Biolabs, Beijing, China), and used as the template for PCR. Three primer pairs were designed to target the RNA-dependent RNA polymerase genes, the glycoprotein genes and N protein genes in the conserved core region of a novel bunyavirus in China (Table 1). The PCR products were analyzed using 2% agarose gel electrophoresis (Figure 2). Then, the products were sequenced with an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The mRNA sequence of these genes deposited in GeneBank (genes of the RNA-dependent RNA polymerase accession No. AB678796, genes of glycoprotein accession No. AB678797 and genes of Nproteinaccession No. AB678798). The sequences of these genes PCR products were compared with known sequences of novel bunyavirus in the GenBank database.

The results of PCR product electrophoresis patterns indicated that the products have identical patterns with SFTSV ones, and the sequence analysis demonstrated that the nucleotide sequences of the RNA-dependent RNA polymerase, the glycoprotein and Nprotein DNA fragments from this case had a 96%, 94% and 95% homology to nucleotide sequences of novel Bunyavirus found in blood samples of patients with SFTS in Central and Northeast China, respectively.

3. Discussion

It is known that SFTS needs to be differentiated clinically from human anaplasmosis, hemorrhagic fever with renal syndrome, and leptospirosis. Patients with human anaplasmosis have fever, but leucocytopenia, thrombocytopenia, and gastrointestinal symptoms are infrequent. Leptospirosis may be confused with SFTS because of the initial fever, chills, headache, myalgia, and abdominal pain, but common symptoms of leptospirosis, such as rash and jaundice (*3*), are rare in patients with SFTS. Most phleboviruses are associated with sand flies, and, in such cases, there is evidence of transdermal transmission. SFTSV RNA was detected in some ticks, indicating that ticks may serve as one of the candidate vectors of SFTSV (*2*).

In this case, the patient was an aged farmer who had been living in the hilly areas of Zhoushan Island, a relatively isolated location in Zhejiang province. The patient presented with high fever and thrombocytopenia accompanied by chills and pharyngeal pain. There was no history of tick bites or contacts with individuals who had similar manifestations. The sanitary condition of her house was poor and it was probably infested by rodents. No similar cases were reported in the village where she lived. According to retrospective investigations of the local medical clinic that the patient first visited, there were about 15 similar cases annually in the past decade, and patients were mostly elderly. Despite the lack of laboratory results, these patients may be identified as probable SFTS cases according to their similar clinical features. Tick bite may be one way of transmission (*1*), and reports by Wenyuan Tao (*4*) of six cases of SFTS occurring in a cluster around a single initial SFTSV patient suggest contact transmission may occur. Further research is required to determine whether there are other routes of transmission.

The laboratory tests of this patient showed that white blood cell and platelet counts decreased progressively during the early stage. The platelet count reached a nadir of 12×10^9/L on the 11th day after the onset of the disease. Liver dysfunction and cardiac dysfunction became more evident. It is known that SFTSV can invade heart, liver, kidney, gastrointestinal tract and other vital organs as well as the bloodstream, as indicated by the elevated levels of serum amylase, lipase, blood glucose and the abnormalities in routine urinalysis. This might be the reason for multiple system

organ failure and deaths caused by severe SFTS (*5*). A review of the current case suggests (*6*), rapid loss of both $CD4^+$ and $CD8^+$ T lymphocytes during the acute phase of the severe respiratory syndrome, which might be associated with the damage of cellular immune function. On the 9th day after the onset of illness, this patient's $CD4^+$ and $CD8^+$ T cells decreased. Thus, it was concluded that the decline of $CD4^+$ and $CD8^+$ T cells may be related to the degree of damage to cell-mediated immune function by the SFTSV. More studies are needed to determine the specific mechanism of this phenomenon.

SFTS was previously identified in six provinces of China, which were Henan, Hubei, Shandong, Anhui, Jiangsu and Liaoning, and surveillance for SFTS was carried out outside of these regions. Our finding indicates that SFTSV is the probable cause of a SFTS case outside of the reported endemic regions. Although this first case of SFTSV caused SFTS found in Zhejiang had no travel history to the above endemic regions of SFTSV in China, the patient lived in a wooded and grassy area with lots of chances for tick exposure and was infected with the existing SFTSV. It is most likely that SFTS had not only been prevalent in the above 6 provinces, but also including Zhejiang Province, which is adjacent to Jiangsu and Anhui. Our findings suggest more research is needed to determine the extent to which this disease occurs, especially in the area near the endemic regions.

SFTS as an emerging infectious disease in China is progressive in nature and potentially fatal. The epidemiology, pathogenesis and route of transmission of this disease are still unclear, the clinical manifestations can be systemic and the treatment is challenging. More emphasis should be given to this disease and further training of medical personnel should be carried out to prevent misdiagnosis, especially in the epidemic areas.

Acknowledgements

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Commentary

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"US-JAPAN CONFERENCE: INFLAMMATION, DIABETES AND CANCER" held at the Beckman Research Institute of City of Hope, Duarte, CA, USA

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Summary **The conference was held to facilitate research collaborations between US and Japan scientists, and to commemorate the rich history of the Beckman Research Institute of City of Hope (COH) research contributed by many Japanese doctors. Most noticeable is Dr. Ryojun Kinoshita, an internationally renowned oncologist who built the first research team at the campus of COH Medical Center in 1952. The conference received enthusiastic support from Junichi Ihara, Consul General of Japan, Los Angeles. During the two day conference, seven scientists from Japan, six scientists from Southern California, and six scientists from COH presented various aspects of cancer and diabetes research which included nuclear receptor regulation, DNA base and chromatin modifications, cancer glycosylation, circadian clock, cell polarity, tumorigenesis, micro and small RNA therapies, genomics, epigenetics, and signaling.**

Keywords: Diabetes, cancer research, Ryojun Kinoshita

The two-day conference (Figure 1A) was held at Argyros Auditorium of the Beckman Research Institute of City of Hope (COH) in Duarte, CA, USA on August 4-5, 2011, with the support of the Consulate General of Japan, Los Angeles. The major aim of the conference was not only to facilitate collaborations between Japanese scientists and COH scientists as well as Southern Californian researchers, but also to commemorate the rich history of COH research which started in 1952 by Japanese doctors at the campus of COH Medical Center. Prior to the conference, a welcome reception was hosted by Junichi Ihara, Consul General of Japan, Los Angeles, at his residence in Hancock Park for conference speakers and invited guests (Figure 1B).

On the first day of the conference, Dr. Arthur Riggs, Chair of Diabetes and Metabolic Diseases Research and Emeritus Director of Beckman Research Institute, presented historic aspects of COH Research (Figure 1C). As seen in the photo on the screen behind Dr. Riggs, the first research team was launched in 1952 by bringing an internationally renowned oncologist, Dr. Ryojun Kinoshita, as Director who was a visiting professor at UCLA at the time. This initial phase of COH research establishment is briefly described at the end of this article. Figure 1D shows the conference center audience listening to Dr. Riggs' presentation, including at the front raw from the right, Dr. Richard Jove (Director of Beckman Research Institute), Dr. Michael Friedman (President and CEO of COH), and the Honorable Junichi Ihara, Consul General of Japan, Los Angeles who gave welcome speeches. The sessions started by a keynote speaker, Dr. Shizuo Akira (Osaka University) (Figure 1E), followed by Dr. Hua Yu (COH), Dr. Christopher Glass (UCSD), Dr. Rama Natarajan (COH), Dr. Peter Jones (USC), Dr. Taeko Miyagi (Tohoku Pharmaceutical University), Dr. Paolo Sassone-Corsi (UCI), Dr. Fumitoshi Ishino (Tokyo Medical and Dental University), and Takahiro

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Figure 1. Event photos. (A) The program, **(B)** Keynote speaker Dr. Akira at the pre-conference reception at Consul Ihara's residence, **(C)** Dr. Riggs talked about Dr. Kinoshita, **(D** and **E)** oral presentations and the audience, **(F)** poster session, **(G)** Keynote speaker Dr. Evans, (**H**) Consul Ihara, **(I)** Dinner reception at the COH Visitor Center, and **(J)** Dr. Itakura talked about Dr. Kinoshita.

Maeda (COH). Poster session and reception were held after the excellent first day presentations (Figure 1F). The poster session was planned to enhance interaction between young Japanese scientists and COH researchers. Most of the invited speakers from Japan brought their associates who presented their research. Three young scientists from Japan presented their posters and introduced their projects to COH researchers and invited speakers. During the reception, Dr. Ronald Evans who gave a keynote lecture on the 2nd day of the conference was greeted by COH scientists, Drs. Shi, Hua, and Jove (Figure 1G).

During COH-hosted dinner at the beautiful COH Visitor Center (Figures 1H-1J), the Honorable Junichi Ihara, Consul General of Japan, Los Angeles, gave a speech to celebrate the occasion of the US-Japan Conference (Figure 1H). Dr. Keiichi Itakura then commemorated the rich history of the early COH research by showing the photo of the first director of COH Research Institute, Dr. Ryojun Kinoshita (Figure 1I), and presenting stories of Dr. Susumu Ohno who joined COH at the beginning and stayed to contribute to cytogenetics and theoretical biology by outstanding discoveries (Figure 1J).

On the 2nd day of the conference, after a great talk by keynote speaker Dr. Ronald Evans, Dr. John Rossi (COH), Dr. Masatoshi Hagiwara (Kyoto University), Dr. Barbara Wold (CALTECH), Dr. Hong Wu (UCLA), Dr. Seishi Ogawa (The University of Tokyo Hospital), Dr. Gerd Pfeifer (COH), Dr. Shigeo Ohno (Yokohama City University Medical School), and Dr. Satoshi Inoue (The University of Tokyo), presented their forefront research findings. Over 200 scientists and graduate students from COH and other institutions fully benefited from the excellent presentations by invited speakers.

The conference thoroughly covered the theme of inflammation, a currently emerging new root for cancer and diabetes. Experts in their own fields presented various aspects of cancer and diabetes research which included nuclear receptor regulation, DNA base and chromatin modifications, cancer glycosylation, circadian clock, cell polarity, tumorigenesis, micro and small RNA therapies, genomics, epigenetics, and signaling as seen in the program.

Acknowledgements

We thank the speakers for sharing their research findings, Beckman Research Institute of the City of Hope, Japan Consulate, and Tokai University for their support. We also thank the meeting staff and organizers for their time and effort. YFY and RJL thank Ms. Faith Osep for her excellent secretarial assistance and Mr. Patrick Cunningham for the professional photography of the conference events.

City of Hope

The Program

Thursday, August 4, 2011

Welcome
Michael A. Friedman, M.D., President and Chief Executive Officer, Cancer Center Director City of Hope
The Honorable Junichi Ihara, Consul General of Japan, Los Angeles

Historic Aspects of City of Hope Research
Arthur D. Riggs, Ph.D., Chair, Diabetes and Metabolic Diseases Research, Beckman Research Institute, City of Hope

SESSION 1: INFLAMMATION

Chair: **Janice Huss**, Ph.D. and **Ravi Bhatia**, M.D., City of Hope

1-1. Keynote Speaker: **Shizuo Akira**, M.D., Ph.D., Immunology Frontier Research Center, Osaka University
Negative regulation of immune responses by the zinc finger domain containing nuclease, Zc3h12a

1-2. Hua Yu, Ph.D., Beckman Research Institute, City of Hope
STAT3 in cancer inflammation and immunity

1-3. Christopher K. Glass, M.D., Ph.D., University of California, San Diego
Macrophages, inflammation and insulin resistance

1-4. Rama Natarajan, Ph.D., F.A.H.A., F.A.S.N., City of Hope
Epigenetics and MicroRNAs in the regulation of inflamatory genes Under diabetic conditions

SESSION II: MOLECULAR ONCOLOGY

Chair: **Susan Kane**, Ph.D. and **Yun Yen**, M.D., Ph.D., City of Hope

2-1. Peter A. Jones, Ph.D., D.Sc., USC Norris Comprehensive Cancer Center
The cancer epigenome

2-2. **Taeko Miyagi**, M.D., Ph.D., Cancer Glycosylation Research Laboratory, Tohoku Pharmaceutical Univeristy
Aberrant expression of sialidase and cancer progression

2-3. **Paolo Sassone-Corsi**, Ph.D., University of California, Irvine
Joining the dots: epigenetics, metabolism, and the circadian clock

2-4. **Fumitoshi Ishino**, Ph.D., Medical Research Institute, Tokyo Medical and Dental University
Role of retrotrasposons in mammalian evolution

2-5. **Takahiro Maeda**, M.D., Ph.D., City of Hope
Endocytic regulation of normal and malignant hematopoiesis by the clathrin assembly protein CALM

Poster Session & Reception

Friday, August 5, 2011

SESSION III: GENE REGULATION

Chair: **Yanhong Shi**, Ph.D. and **Toshifumi Tomoda**, M.D., Ph.D., City of Hope

3-1. Keynote Speaker: **Ronald M. Evans**, Ph.D., Salk Institute for Biological Studies
Nuclear receptor regulation of the inflammatory cistrome

3-2. **John Rossi**, Ph.D., City of Hope
Small RNA therapies for HIV and cancer

3-3. **Masatoshi Hagiwara**, M.D., Ph.D., Graduate School of Medicine, Kyoto University
Visualization of alternative splicing and the therapeutic manipulation with chemical compounds

3-4. **Barbara J. Wold**, Ph.D., California Institute of Technology
Genome-wide chromatin modification status and transcription factor occupancy in differentiating systems:Relating physical connectivity to biological function

SESSION IV: GENOMICS AND SIGNALING

Chair: **Piroska Szabo**, Ph.D., City of Hope and **Nozomu Mori**, Ph.D., Nagasaki University

4-1. **Hong Wu**, M.D., Ph.D., University of California, Los Angeles
PTEN and Tumorigenesis

4-2. **Seishi Ogawa**, M.D., Ph.D., The University of Tokyo Hospital
Genetic analysis of myelodysplastic syndromes and related disorders

4-3. **Gerd Pfeifer**, Ph.D., City of Hope
DNA base modifications in human cancer

4-4. **Shigeo Ohno**, Ph.D., Yokohama City University School of Medicine
Cell polarity and the maintenance and disruption of epithelial tissues

4-5. **Satoshi Inoue**, M.D., Ph.D., Graduate School of Medicine, The University of Tokyo
Genome-wide androgen receptor signaling in prostate cancer

CLOSING REMARKS

Yoko Fujita-Yamaguchi, Ph.D., Tokai University School of Engineering

Prof. Ryojun Kinoshita, M.D. (1893-1977)

— An oncologist who started the research department at City of Hope

In 1952, Dr. Kinoshita who was then a visiting professor at UCLA was appointed as the director to take on the challenging task of developing cancer research at City of Hope Medical Center. City of Hope originally started as a sanatorium for Jewish tuberculosis patients in 1913 in a small town called Duarte in the Los Angeles suburb. Dr. Kinoshita was recommended by Prof. Charles Carpenter, who was Chairman of the Department of Infectious Diseases in the new Medical School at UCLA. Prior to coming to the US in 1949, Dr. Kinoshita was professor at Hokkaido and Osaka Imperial Universities, and became the first dean of the new Osaka City University Medical School. He organized three consecutive annual meetings of Japanese Cancer Association (1947-1949) as the president of the association. Dr. Kinoshita, when he was at Osaka University, visited the US as a guest of the American Cancer Association and achieved fame as the discoverer of "butter-yellow" cancer in rats fed with a yellow dye.

Dr. Kinoshita asked Susumu Ohno (D.V.M., Ph.D.) to join City of Hope, who was a post-doctoral fellow under the mentorship of Prof. Charles Carpenter at UCLA. Dr. Ohno was instrumental in the drive to further cytogenic research and to develop forefront biology programs. Later in 1982, he was elected to be a member of the National Academy of Sciences in recognition of his work on X chromosome inactivation and his theory of evolution by gene duplication. Dr. Eugene Roberts joined City of Hope as the chairman of Department of Biochemistry in 1954. During 1960s, the Divisions of Biology and Neurosciences headed by both scientists, respectively, were created. Dr. Roberts has been a member of the National Academy of Sciences since 1988 in recognition of his discovery of GABA which is a major inhibitory neurotransmitter in the vertebrate central nerve system, and is now Director Emeritus. Dr. Roberts is 91 years old and he actively participated at the US-Japan Conference. The Division of Immunology was established in 1972 by Dr. Charles Todd. Dr. Kinoshita had successfully accomplished his mission during his tenure. Dr. Kinoshita spent some years of his early research training in Cambridge and married an English woman. He had a manner as an English gentleman with an excellent command of English. Mrs. Kinoshita passed away in 1971 at City of Hope Medical Center. Dr. Kinoshita died in 1977 of a stroke after several years of suffering. He was survived by Ms. Akiko Lotspiech, Dr. Kinoshita's niece and an adopted daughter.

Prof. Ryojun Kinoshita, M.D. (1893-1977)

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