Cytogenetic biodosimetry intercomparison exercises among laboratories in South Korea

Yang Hee Lee[1], Younghyun Lee[1], Hyo Jin Yoon[1], Ki Moon Seong[1]

1 Laboratory of Biodosimetry, National Radiation Emergency Medical Center, KIRAMS, Seoul, Korea

Abstract

In order to increase capacity of biodosimetry performance in large scale radiological accidents, standardized protocols and quality assurance of biodosimetry laboratories within networks are required. An intercomparison exercise is a useful tool to validate the performance of laboratories and harmonize the protocols. We launched an intercomparison exercise to harmonize scoring protocol and develop an image repository for education and training. Participating laboratories shared metaphase images previously generated for dicentric and translocation assay. Total 3000 metaphase images were scored according to their own scoring method first. The scoring results and sheets were shared and inconsistent images were re-evaluated. Metaphase images with agreement were compiled to an image repository, and inconsistent images were used to harmonize scoring protocol. Key issues were analysis of chromosomes with centromeres in terminal position and twisted chromosomes, nomenclature for translocation analysis. This study found the issues required for harmonizing scoring criteria and maintain comparable capacity, which would be further discussed and harmonized in next exercises. This could provide valuable knowledge for standardization of scoring for biodosimetry worldwide, which would further enhance the capacity of national and international biodosimetry net works.

Background

Biodosimetry is an important tools for measuring the absorbed dose of individuals exposed to radiation using biomarkers obtained from human in the events of a mass casualty accidents. However, It takes the extensive time and expertise to analyze aberration chromosomes, so in order to supplement this, it is necessary to operate an inter-institutional network and build a cooperative system for mass casualty accidents. In Korea, three institutions are currently conducting biological dose assessment for radiation exposure, but the cooperative system between institutions is insufficient and the level of dose assessment technology is not well evaluated, making it difficult to jointly respond to radiation accidents. Therefore, we launched an intercomparison exercise to validate the performance of biodosimetry laboratories and discuss the standardization of scoring criteria in the Korea biodosimetry network.

Materials & Methods

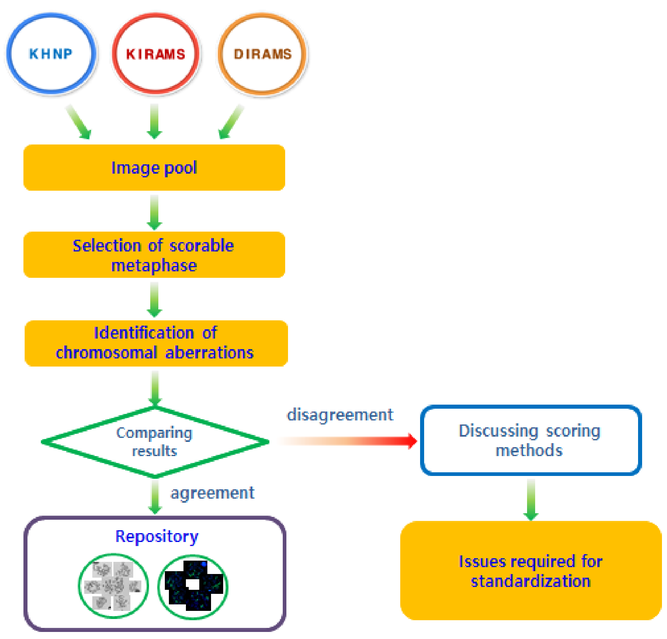

Figure 1. Schematic representation of an intercomparison exercise in South Korea.

Image generation and distribution

Each laboratory shared metaphase images previously generated for DCA and FISH-based translocation assays (*n*=500 each) with other participating laboratories for this exercise. DCA and FISH-based translocation assays were performed according to their own protocols based on the guidelines recommended by the International Atomic Energy Agency (IAEA) and International Organization Standardization (ISO) (IAEA 2011; ISO 2014a, 2014b, 2019). Image files captured with Metafer 4 Autocap software were sent to each laboratory. A total of 3000 (*n*=1500 each for DCA and FISH assays) images were shared with participating laboratories.

Scoring aberrations and comparison

Each laboratory marked scorable metaphases, dicentrics, and translocations according to its own scoring protocol. Translocations were scored in only stable cells without unstable aberrations such as dicentrics, rings or acentrics. Each scoring sheet was shared with other laboratories. When the scoring result was not consistent between participating laboratories, each laboratory recorded their results and described how to score. Metaphase images with consistent scoring were compiled in an image databank, and images with inconsistent results were discussed in a technical workshop.

Statistical analysis

Statistical analyses were performed using GraphPad Prism ver.9 software (GraphPad software, San Diego, CA, USA). Differences between laboratories were analyzed using oneway ANOVA. Two-tailed p value less than .05 was considered statistically significant.

A technical workshop for an intercomparison exercise

A workshop for this intercomparison exercise was held in September 2020. Detailed scoring protocols of each laboratory were introduced, including the number of centromeres, identification of chromosome alterations (e.g. centric rings, acentrics, chromosome breakage, fragments), rejection of metaphases, and nomenclature. Methods used to score metaphase images with inconsistent results were compared and discussed in depth, as well as several ideas for reducing scoring variation.

Result

Table 1. Comparison of scoring dicentrics and translocations among biodosimetry labs.

		Dicentric chromosome assay		Translocation assay	
		% Scorable metaphases	Frequency of dicentrics/cell	% Scorable metaphases	Frequency of translocations[c]/cell
Set A[a]	Lab A	98.4	0.650	85.0	0.508
	Lab B	95.8	0.626	67.6	0.512
	Lab C	98.0	0.627	80.8	0.508
	Mean ± SD	97.4 ± 1.4	0.634 ± 0.014	77.8 ± 9.1	0.509 ± 0.002
	CV (%)[b]	1.4	2.1	11.7	0.5
Set B[a]	Lab A	90.2	0.353	80.8	0.032
	Lab B	91.4	0.333	79.6	0.033
	Lab C	91.2	0.368	84.2	0.031
	Mean ± SD	90.9 ± 0.6	0.351 ± 0.018	81.5 ± 2.4	0.032 ± 0.001
	CV (%)[b]	0.7	5.0	2.9	3.1
Set C[a]	Lab A	85.6	0.154	68.2	0.106
	Lab B	89.2	0.168	67.8	0.097
	Lab C	91.6	0.177	70.0	0.091
	Mean ± SD	88.8 ± 3.0	0.166 ± 0.012	68.7 ± 1.2	0.098 ± 0.008
	CV (%)[b]	3.4	7.0	1.7	7.7

[a]Image sets A, B, and C were provided by Lab A, B and C, respectively.
[b]CV: coefficient of variation.
[c]Reciprocal translocations t(Ab) + t(Ba) and non-reciprocal patterns t(Ba) + ace(b), t(Ab), and t(Ba) were considered.

Table 2. Summary of scoring discrepancies in biodosimetry labs.

Assay	Category	Inconsistent issues	Current consensus in Korea biodosimetry network
DCA	Selection of scorable metaphases	1. No. of acceptable centromeres for scorable metaphases is different. (i.e. A and C: 45 more centromeres, B: 45 and 46 centromeres)	Will be discussed in next technical workshop
	Identification of dicentrics	1. Identification of dicentrics, including chromosomes with centromeres in a terminal position (D and G group), is controversial. 2. Distinction between twisted chromosomes and dicentrics is unclear.	There is a need to understand morphology of D and G group chromosomes for accurate scoring. The twisted region is darker than dicentrics.
	Identification of fragments	1. Classification of fragments, acentric rings, and minutes is unclear.	Will be discussed in next technical workshop
FISH	Selection of scorable metaphases	1. The kinds of unstable aberrations to be rejected are different. (A and C: dicentrics, centric rings, fragments, acentric rings, or minutes; B: dicentrics, centric rings, fragments, or acentric rings). 2. It is controversial to include cells with signal loss or addition.	Double minutes—small fragments of extrachromosomal DNA—are unstable chromosomal aberrations, which should be excluded for scoring translocation. Cells with signal loss should be excluded.
	Identification of translocations	–	–
	Recording translocations	1. Translocations and related aberrations are recorded according to each lab's own nomenclature. 2. The needs of differentiation between two- and one-way translocation are unclear.	Will be discussed in next technical workshop

DCA: dicentric chromosome assay; FISH: fluorescence *in situ* hybridization.

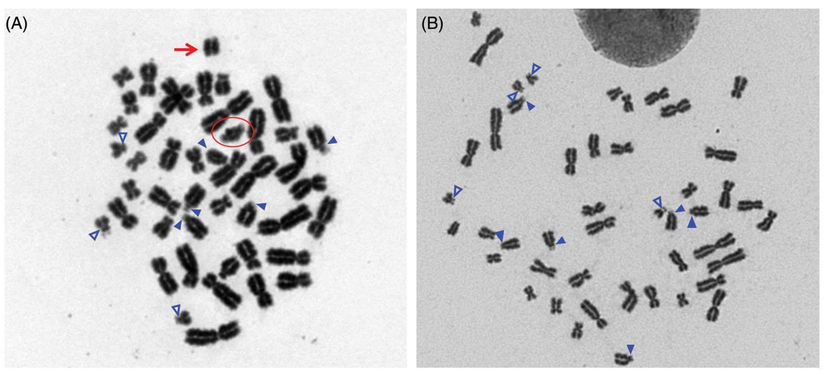

Figure 2. A representative metaphase image containing dicentrics consisting of chromosomes with centromeres in terminal position. (A) Circle and arrow indicate dicentrics and fragment, respectively. One laboratory reported that the dicentric consists of D or G group chromosomes, whereas another laboratory considered it a normal chromosome. (B) Normal metaphases containing three pairs of D group and two pairs of G group chromosomes are shown. Closed and open triangles indicate D and G group chromosomes, respectively.

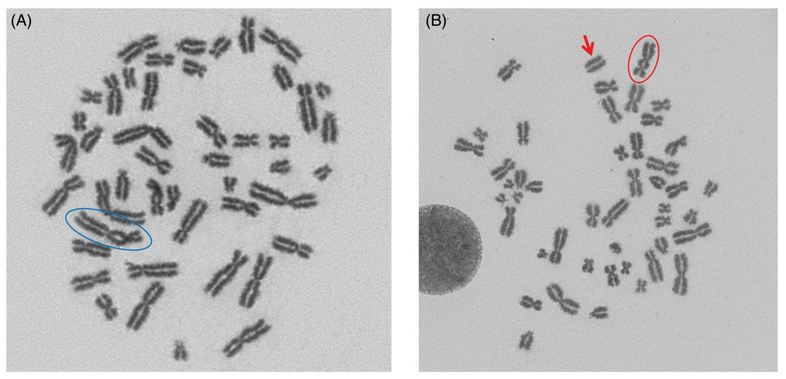

Figure 3. Representative metaphase images containing twisted chromosomes and normal chromosomes. (A) A circle indicates a twisted chromosome with a dark region where it is twisted, unlike dicentrics. (B) Typical type of dicentrics and fragments are shown. Circle and arrow indicate dicentrics and fragments, respectively.

Conclusions

We performed an intercomparison exercise to compare scoring criteria for biodosimetry in South Korea. The knowledge gained from an extensive discussion of scoring discrepancies may contribute to recommendations for standardization of DCA and FISH assays across biological laboratories worldwide. This kind of exercise could be expanded to a regional (e.g. ARADOS) and international biodosimetry network. One laboratory is a member of the ARADOS network and has also obtained accreditation under ISO 15189:2012. IAEA has suggested that new biodosimetry laboratories should be organized and maintained in agreement with the IAEA guidelines and ISO documents for quality assurance and controls (Vinnikov and Belyakov 2020). A combination of ISO accreditation and standardized protocols would enable biodosimetry laboratories to provide reliable and comparable dose assessment, with appropriate quality controls, which would reinforce the mutual cooperation between biodosimetry laboratories in large-scale radiological accidents.

This study was supported by grants from the Korea Institute of Radiological and Medical Sciences (No. 50445-2021) and the National Research Foundation of Korea Grant (No. NRF2019R1A2C208741613), funded by the Ministry of Science and ICT, Republic of Korea.

KOREA INSTITUTE OF RADIOLOGICAL & MEDICAL SCIENCES