·综述·

Learning From Biomarkers in Victims Accidentally Exposed to Ionizing Radiation

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【**Abstract**】 Biomarkers, such as chromosome aberration and micronuclei assays, prove to be reliable for facilitating clinical diagnosis in radiation accidents. In a radiation accident in India, chromosomal aberration, γ-H2AX, as well as other blood markers, were detected in accidentally exposed victims. This multi-parametric approach aided in confirming that individuals had been exposed by ionizing radiation. However, doses were impossible to estimate because of a 30-day delay in accident awareness. Exposure dose for victims was estimated using a dose-response curve previously established. Dose estimation, blood cell depletion kinetics, and no appearance of prodromal symptoms suggested that doses of exposure were low. Hematologic investigation, sampling time, and chromosome aberration scoring were all proposed according to data from the victims exposed to ^{60}Co. Finally, knowledge regarding chromosome aberration analysis and the importance of international co-operation and assistance should be shared from this accident.

【**Key words**】 Radiation, ionizing; Accident; Chromosome aberration; Lessons

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Ionizing radiation is a known inducer of cytogenetic abnormalities including chromosome aberrations and micronuclei in human peripheral lymphocytes. Accurate dose estimates can be made by biological dosimetry to predict acute radiation syndrome (ARS) within days after a radiation accident or a malicious act involving radiation[1-2]. Timely information on dose is quintessential for the medical management of acutely irradiated personnel. Chromosome aberration is widely used as a sensitive biomarker for evaluating the damage caused by acute radiation exposure[3-4]. Specifically, dicentric chromosomes (dic) and rings (r) are standard markers for radiation exposure[5-6]. Moreover, cytokinesis-blocked micronuclei (CBMN) supplement chromosome aberration analysis[7]. A single biological assay cannot fully evaluate biodosimetry requirements in complex exposure scenarios. Recent studies are currently focused on searching for new biomarkers for radiation damage evaluation and dose estimation. Research on multifaceted methods for biological assessments seem to aid in clinical management of radiation accident victims[8].

1 Biological dosimetry for radiation accidents

Biological dosimetry, based on the analysis of chromosomal aberrations (dic+r), has been used for

more than 50 years and has become the golden standard test for dose estimation in the past radiological accidents[9]. In case of a radiation accident, the first information comes especially from physical dose reconstruction, blood count data, and from the clinical symptoms that exposed persons might display. Undoubtedly, all the information may be combined with the results of biological dose assessments to obtain a clearer diagnosis of the exposed persons. Biological dosimetry using cytogenetic methods is of particular importance because it considers inter-individual variation in susceptibility to radiation damage. Thus, many basic and clinical studies have found that there was a close relationship between dic+r chromosomal aberrations induced in peripheral blood lymphocytes (PBLs). This relationship allows dose estimation of an accidentally exposed person by comparing the observed aberration yield of dic+r to an in vitro biological-dose curve. The power of dic+r for dose estimation is related to the low and constant spontaneous aberration rate in the healthy population (about 1‰) and by the fact that dic+r are special in radiation induced damage[10]. Biological doses down to 0.1 Gy can be detected by chromosomal aberration after whole-body irradiation (WBI) by low linear energy transfer(LET) radiation. However, in cases of exposure by low dose radiation, the disadvantage of dic+r assay is the time needed for microscopic scoring analysis of abundant number of metaphase cells.

Dic+r assay performed in PBL was the only method available for many years, and it is also the gold standard for cytogenetic radiation dosimetry until now. However, a number of additional assays was available and validated in the past years, including micronucleus(MN), translocation, and premature chromosome condensation assays[11].

Previous studies established the feasibility of using geographically dispersed laboratories to provide accurate dose estimates from samples originating in one location and being shipped around the world for processing and analysis using the dicentric assay[12]. In addition, an analysis of 50 metaphases provids very reliable and accurate estimations of individual doses over a range of 0.75–4.5 Gy, a most are within 20% of the range of the applied doses. Even dose estimations based on analysis of only 30 metaphases and even 20 metaphases allow an accurate evaluation.

2 Analysis of biological dose after radiation accident

Interestingly, Gupta et al.[13] described blood biomarkers in a ^{60}Co radiation accident in India. They detected chromosome aberration, γ-H2AX, and other blood parameters including total leukocyte counts and platelet counts in the victims. This multi-parametric approach confirmed that individuals exposed, providing valuable information for assessment and management of victims for radiation accidents in future. Work of Gupta et al. included valuable data on blood parameters, dicentrics, γ-H2AX, and clinical symptoms in victims exposed accidentally[13].

2.1 Hematology investigation for the victims

Physicians should determine leukocyte, platelet, and haemoglobin levels daily, especially after hospital admission. Calculating daily blood cell depletion kinetics is essential for medical management of radiation victims. For scientific interest purposes, the laboratory should obtain blood samples at frequent intervals to monitor changes in differential white cells.

2.2 Sampling time for chromosome aberration

For the cytogenetic assay, venipuncture blood samples should be taken within four weeks after exposure. After this period, aberration yields appear to decline, causing greater uncertainty in radiation dose estimation[6]. We obtained blood samples 4 and 56 days after a ^{60}Co accident in China, for three victims, which showed that sampling time for dose estimation using chromosome aberration is no more than 56 days after exposure[14] (Table 1). Thus, radiation dose estimation for Indian victims is acceptable after 30 days after the incidence.

2.3 Results of chromosome aberration

Results of chromosome aberration for patient 4 (P4) in Table 1 were inconsistent with those in Fig.3 of

Table 1 Chromosome aberration analysis and biological dose estimation 4 and 56 days post exposure

Subject	Sex	Age (years)	4 days post exposure		56 days post exposure	
			Metaphase studied (dic+r)	Dose, Gy (95% *CI*)	Metaphase studied (dic+r)	Dose, Gy (95% *CI*)
A	F	38	40(79)	5.09 (4.46–5.64)	150(357)	5.61 (5.24–5.95)
B	M	8	217(113)	2.49 (2.23–2.74)	300(154)	2.48 (2.26–2.68)
C	M	37	334(188)	2.61 (2.40–2.80)	300(178)	2.68 (2.46–2.89)

Notes: dic+r: dicentric chromosomes and rings; *CI*: confidence intervals.

Cupta et al.[13]. Two dicentrics in 43 metaphase lymphocytes of P4 are recorded in Table 1, but approximately 20 dicentrics were found according to dicentric frequency 30 days after exposure. This discrepancy may be due to dose estimation differences between dicentric frequencies for P4. Based on data in Table 1 (Gupta et al.)[13], we estimated the dose of five victims using the dose-response curve[14]of γ ray-induced chromosome aberration(Table 2):

$$Y1=3.4967\times10^{-2}D+6.9490\ 7\times10^{-2}D^2 \quad (1)$$

Where $Y1$ denotes dic+r number in each lymphocyte and D is the radiation dose(Gy). The dose range is 0.5 Gy to 5.0 Gy. The mean radiation dose and its 95% confidence interval were thus calculated for each subject based on Equation(1).

Table 2 Biological dose estimation based on Gupta et al. (30 days post exposure)

Subject	Metaphase studied	Dicentrics	Mean dose, Gy(95% *CI*)
P1	35	2	0.69(0.00–1.16)
P2	30	2	0.76(0.00–1.27)
P3	62	1	0.29(0.00–0.61)
P4	43	2	0.61(0.00–1.03)
	43	20[a]	2.35(1.89–2.73)
P5	57	2	0.50(0.00–0.87)

Note: a: estimation according to frequency of dicentric in Fig. 3 by Gupta et al.; *CI*: confidence interval.

According to estimated doses in Table 2, five victims may suffer from mild acute radiation sickness (ARS), but P4 may suffer from moderate ARS if 20 dicentrics is the correct chromosome aberration score. On the 30th day after the accident, the total leukocyte counts in patients P1, P2, P3 and P5 ranged from 3100–5600/mm[3], but only 30–62 metaphase lymphocytes were found in chromosome aberration analysis, which may be the reason for the failed radiation dose estimation.

Additionally, a dicentric was not marked in Fig.4 of Gupta et al.[13](Fig.1). Reliable scoring of chromosome aberrationwas a dependable means for dose estimation and offers valuable information for treatment of ARS patients; its omission might result in the underevaluation of radiation dose.

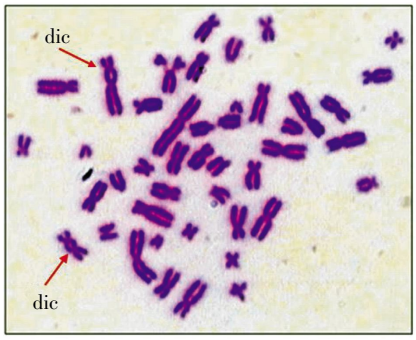

Fig.1 Chromosome aberration of an Indian accident victim. Notes: red arrow show two dicentric chromosomes.

3 Lessons to be learned in dose estimation

Blood cell depletion kinetics and absence of prodromal symptoms suggested that the radiation exposure dose was not very high. However, one patient (P4) died of multiple organ failure. This finding may be attributed to delayed clinical management and protracted radiation exposure. Gupta et al.[13] reported insight into the preparation, assessment, and management of such accidents. We report retrospective lessons learned.

First, reliable chromosome aberration analysis provides valuable information regarding damage and dosimetry, facilitating clinical diagnosis. Chromosome aberration analysis is widely accepted as the gold standard biomarker for radiation damage evaluation and dose reconstruction following acute radiation exposure[6]. In a similar accident in December 1998 in Istanbul, with respect to radiation source and approximate radiation dose level, chromosome aberration analysis was shown to be critical for estimating radia-

tion dose and facilitating an accurate clinical diagnosis[8]. In the Istanbul accident, time lapse between the incident and recognition by authorities was over a month, but the number of metaphase lymphocytes in chromosome aberration analysis was 700–1300. Successful dose estimation provided pertinent data for clinical triage and therapy for the 10 victims.

Second, international cooperation and communication is vital in radiation accidents. Convention on Assistance in the Case of a Nuclear Accident or Radiological Emergency sets an international framework for cooperation among the State and International Atomic Energy Agency(IAEA) to facilitate prompt assistance and support in nuclear accidents or radiological emergencies[15]. The IAEA is the focal point for such cooperation through channeling information, supporting efforts, and providing available services. Assistance provided by IAEA includes technical advice on emergency planning, preparedness and response, assistance with radiological surveys and retrieval of sources, assistance with in situ verification of radiological conditions and technical advice, and medical advice for overexposed persons[16]. The IAEA has provided support and assistance in serious accidents involving radiation sources for many years. Technical support may have greatly benefited from biological dose estimation and medical management for Dehli victims if Indian authorities requested assistance from the IAEA in obtaining advice.

4 Prospections about biological dose estimation

A comparison among the lab curves demonstrated that a significant difference exists between the coefficients of the curves in some laboratories[17–19]. The differences in dose rate could explain this to some extent, as the pre-curve from different labs were made from radiation with the highest dose rate and had the largest difference among the curves. However, dose rate only partially accounts for these differences, as demonstrated by curves from many of the labs, although it did not have the same dose rate[20–21]. Based on the original inter-laboratory comparison, calibration curves made in different laboratories can differ even when the same samples are used to generate the curve. These variations can be attributed to factors such as culture conditions, slide preparation, metaphase cell selection and scoring, all of which can potentially outweigh differences in dose rate as long as the irradiation for each dose is delivered within the recommended 15 min[6].

The establishment of an international network, including several cytogenetic reference laboratories, establishes and optimizes International Standardization Organization(ISO) standards for the conventional and automated cytogenetic assay. By the creation of such a network of trained laboratories using similar equipment for cytogenetic automation and the same classifiers, standardized fixation protocols, and so on, comparable results can be obtained, and the throughput of automated dic+r and MN scoring can be increased to allow a rapid response to large-scale radiation accidents. A European program has been started whereby multi-disciplinary biodosimetry tools, including the dic+r and MN assay, will be developed in 15 European groups, to manage high-scale radiological casualties and to increase European capabilities in radiological incident response. The similar program is also needed all over the world.

Further refinement of the cytogenetic assay is needed to optimize its use in retrospective biodosimetry and for the analysis of cases of protracted exposure and partial body exposure. To date, only limited and diverse data are available about the disappearance of cytogenetic assay, and further research and validation is needed. Appropriate calibration curves also need to be established for more complex exposure scenarios.

Conflict of Interest The authors declare no conflicts of interest.

Authors contribution statement Yan Wang and Qiang Liu analyzed and interpreted the data and wrote the paper. Liqing Du and Chang Xu analyzed the data and revised the draft. Qin Wang collected the references and data of this analysis. Zhiyi Song analyzed the communication lessons from nuclear accidents. Jianxiang Liu and Xu Su analysed and interpreted the data, and revised the article critically for important intellectual content. All the authors gave final approval of the

paper to be published.

References

[1] Agrawala PK, Adhikari JS, Chaudhury NK. Lymphocyte chromosomal aberration assay in radiation biodosimetry[J]. J Pharm Bioallied Sci, 2010, 2(3): 197–201. DOI: 10.4103/0975–7406.68501.

[2] Sasaki MS. Cytogenetic biomonitoring of human radiation exposures: possibilities, problems and pitfalls[J]. J Radiat Res, 1992, 33Suppl: 44–53. DOI: 10.1269/jrr.33.Suppl_1.44.

[3] Lloyd DC, Edwards AA. Biological dosimetry after radiation accidents//Obe G , Natarajan AT, Eds. Chromosomal Aberrations: Basic and Applied Aspects[M]. Berlin: Springer, 1990.

[4] Samarth RM, Samarth M, Matsumoto Y. Utilization of cytogenetic biomarkers as a tool for assessment of radiation injury and evaluation of radiomodulatory effects of various medicinal plants-a review [J]. Drug Des Devel Ther, 2015, 9: 5355–5372. DOI: 10.2147/DDDT. S91299.

[5] Thierens H, De Ruyck K, Vral A, et al.Cytogenetic biodosimetry of an accidental exposure of a radiological worker using multiple assays[J]. Radiat Prot Dosimetry, 2005, 113(4): 408–414. DOI: 10.1093/rpd/nch483.

[6] International Atomic Energy Agency. Cytogenetic analysis for radiation dose assessment: A Manual. Technical reports series No 405 [M]. Vienna: International Atomic Energy Agency, 2001.

[7] Vral A, Fenech M, Thierens H. The micronucleus assay as a biological dosimeter of in vivo ionising radiation exposure[J]. Mutagenesis, 2011, 26(1): 11–17. DOI: 10.1093/mutage/geq078.

[8] International Atomic Energy Agency. The radiological accident in Istanbul[M]. Vienna: International Atomic Energy Agency, 2000.

[9] Singh VK, Newman VL, Romaine PL, et al. Use of biomarkers for assessing radiation injury and efficacy of countermeasures[J]. Expert Rev Mol Diagn, 2016, 16(1): 65–81. DOI: 10.1586/14737159.2016.1121102.

[10] Leonard A, Rueff J, Gerber GB, et al. Usefulness and limits of biological dosimetry based on cytogenetic methods[J]. Radiat Prot Dosimetry, 2005, 115(1/4): 448–454. DOI: 10.1093/rpd/nci061.

[11] Blakely WF, Carr Z, Chu MC, et al. WHO 1st consultation on the development of a global biodosimetry laboratories network for radiation emergencies (BioDoseNet)[J]. Radiat Res, 2009, 171(1): 127–139. DOI:10.1667/RR1549.1.

[12] Romm H, Wilkins RC, Coleman CN, et al. Biological dosimetry by the triage dicentric chromosome assay: potential implications for treatment of acute radiation syndrome in radiological mass casualties[J]. Radiat Res, 2011, 175(3): 397–404. DOI: 10.1667/RR2321.1.

[13] Gupta ML, Srivastava NN, Dutta S, et al. Blood biomarkers in metal scrap workers accidentally exposed to ionizing radiation: a case study[J]. Hum Exp Toxicol, 2013, 32(12): 1311–1322. DOI: 10.1177/0960327113482477.

[14] Liu Q, Cao J, Wang ZQ, et al. Dose estimation by chromosome aberration analysis and micronucleus assays in victims accidentally exposed to ^{60}Co radiation[J]. Br J Radiol, 2009, 82(984): 1027–1032. DOI: 10.1259/bjr/62484075.

[15] International Atomic Energy Agency. Convention on assistance in the case of a nuclear accident or radiological emergency[M]. Vienna: International Atomic Energy Agency, 1986.

[16] International Atomic Energy Agency. The radiological accident in nueva aldea[M]. Vienna: International Atomic Energy Agency, 2009.

[17] Wojcik A, Gregoire E, Hayata I, et al. Cytogenetic damage in lymphocytes for the purpose of dose Reconstruction: a review of three recent radiation accidents[J]. Cytogenet Genome Res, 2004, 104(1/4): 200–205. DOI: 10.1159/000077489.

[18] Liu Q, Cao J, Liu Y, et al. Follow-up study by chromosome aberration analysis and micronucleus assays in victims accidentally exposed to ^{60}Co radiation[J]. Health Phys, 2010, 98(6): 885–888. DOI: 10.1097/HP.0b013e3181c4b9c1.

[19] Feng L, He L, Wang Y, et al. Eight-year follow-up study of three individuals accidentally exposed to ^{60}Co radiation: Chromosome aberration and micronucleus analysis[J]. Mutat Res, 2015, 784–785: 10–14. DOI: 10.1016/j.mrgentox.2015.04.005.

[20] Plamadeala C, Wojcik A, Creanga D. Micronuclei versus Chromosomal Aberrations Induced by X-Ray in Radiosensitive Mammalian Cells[J]. Iran J Public Health, 2015, 44(3): 325–331.

[21] Robertson A, Allen J, Laney R, et al. The cellular and molecular carcinogenic effects of Radon exposure: a review[J]. Int J Mol Sci, 2013, 14(7): 14024–14063. DOI: 10.3390/ijms140714024.

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