Radiation Protection Dosimetry

EPR Biodose 2022 Meetings (Official meetings of the IABERD)

Virtual Meeting, March 28-30, 2022,
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In-Person Paris Meeting, June 7-10, 2022,
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Radiation Protection Dosimetry, 2023, **199(14)**, 1565–1571
https://doi.org/10.1093/rpd/ncad053
Paper

A preliminary report on retrospective dose assessment by FISH translocation assay in FDNPP Nuclear Emergency Worker Study (NEWS)

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Abstract

In Japan, a national project of longitudinal health care and epidemiological research (NEWS) was developed in 2014 to analyse the effects of radiation on human health for workers who responded to the Fukushima Dai-ichi nuclear emergency in 2011. In 2018, peripheral blood for chromosome translocation analysis was collected from 62 workers. Retrospective dose assessment was performed with fluorescence *in situ* hybridisation translocation (FISH-Tr) assay. The range of estimated doses by FISH-Tr assay was 0–635 mGy, in which 22 workers had estimated doses of more than 189 mGy. Biological dose estimates were five times higher in workers with physically measured total exposure recordings above 70 mGy. It is likely that smoking and medical exposure caused the discrepancy between estimated biological and physical total exposure doses. Thus, there is a possibility that retrospective biodosimetry assessment might over-estimate occupational exposures to workers exposed to chronic radiation during nuclear emergency work.

Introduction

The Great East Japan Earthquake occurred on 11 March 2011. This earthquake and subsequent tsunami caused serious damage to the Fukushima Dai-ichi Nuclear Power Plant (FDNPP), resulting in the release of large amounts of radioactive materials into the environment. For nuclear emergency work at the FDNPP, the emergency exposure limit was raised from 100 to 250 mSv during the period from 14 March to 16 December 2011. Approximately 20 000 workers engaged in the nuclear emergency work, among which 174 workers received 100 mSv or more from occupational exposure to ionising radiation. A national project of longitudinal health care and the Epidemiological Study of Health Effects in Fukushima Nuclear Emergency Workers (NEWS) has been conducted since 2014 (project leader: Toshiteru Okubo, National Institute of Occupational Safety and Health, Japan; grant: Ministry of Health, Labour and Welfare, Japan)[(1)].

As part of the NEWS project, the biodosimetry team is currently performing retrospective dose assessments in order to support physical dose assessments and clinical findings. In general, the biological half-life of chromosome aberrations is shorter in unstable than stable aberrations because the type of aberration influences

Received: June 30, 2022. **Revised:** January 30, 2023. **Editorial decision:** February 2, 2023. **Accepted:** February 2, 2023

cell cycle progression and apoptosis. The gold standard cytogenetic biodosimetry marker, dicentric chromosome, is an unstable aberration with a shorter biological half-life and is useful for acute radiation dose assessments[2]. On the other hand, translocations are stable chromosome aberrations with a much longer biological half-life. Hence, dose estimation using chromosome translocations as an endpoint is applicable for past radiation exposures[3–5]. In this preliminary study, as peripheral blood was collected from 62 emergency workers seven years after the 2011 emergency work, retrospective biodosimetry with translocations detected with fluorescence *in situ* hybridisation (FISH-Tr) was assessed.

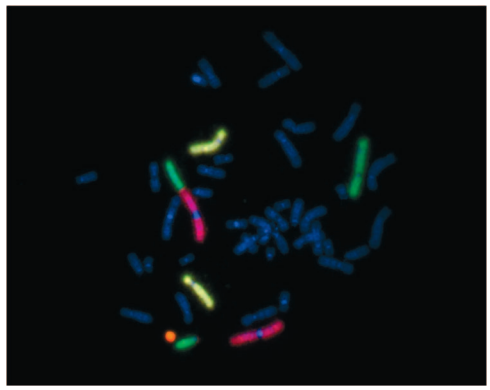

Figure 1. The image of metaphase with a reciprocal translocation involving chromosomes 1 and 2.

Materials and methods

This study was performed as a part of the NEWS project. The NEWS project was established at the Radiation Effects Research Foundation in April 2014, and then was moved to the National Institute of Occupational Safety and Health in April 2019. Furthermore, chromosome aberration analysis was carried out with the approval of the Ethics Committee of the National Institutes for Quantum Science and Technology (Approval numbers: 18-016, 18-019). Informed consent was obtained from all participants.

Exposure and information of emergency workers

For occupational workers, the occupational dose from March to 16 December 2011 was defined as 'emergency exposure', whereas the cumulative dose up until the time of blood sampling was defined as the 'total exposure'. In addition, the sum of the effective internal dose measured by whole-body counters and the external dose measured by personal thermoluminescent dosemeters was used to determine the total exposure. Furthermore, smoking habits and the number of CT and PET-CT examinations were investigated in the NEWS health surveillance as factors that could influence biological dose assessment.

Blood collection and culture

In 2018, peripheral blood from 62 workers who engaged in emergency work at FDNPP in 2011 was collected with their informed consent for chromosome translocation analysis. Peripheral blood mononuclear cells (PBMCs) were isolated from 3-mL whole blood with CPT tubes (BD, BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions, and cultured for 48 h in 5% CO_2 in the presence of phytohemagglutinin-HA15 (Remel Europe, Dartford, UK) and colcemid (Wako, Osaka, Japan). The cultured PBMCs were harvested by a slightly modified method from the IAEA protocol[6]. Briefly, cells were treated with 75-mM potassium chloride at 37°C for 20 min and fixed in cold 3:1 methanol/glacial acetic acid three times. The fixed cells were spread on pre-cleaned microscope glass slides for chromosome translocation analysis.

Chromosome translocation assay

Retrospective dose assessment was performed with FISH-Tr assay using whole-chromosome painting probes for chromosomes 1 (red), 2 (green) and 4 (red + green) (Metasystems, Altlussheim, Germany) (Figure 1)[7]. Genome equivalence correction was performed. Poisson distribution was verified with *u*-test in GOF Poisson[8, 9]. Biological dose was estimated with the 'radir' package[10] in R, using the individual worker's age-matched dose-response curve (DRC) constructed by Abe[11] and Goh[12] (i.e. C values in the DRC equation were modified by adding each worker's background translocation frequency associated with age derived from Sigurdson's equation[14]) and observed translocations. Dose estimates were only reliable if the observed translocations were more than the decision threshold[7].

Results and discussion

Comparison of physical calculated dose and biological estimated dose

The frequency of chromosome translocations in the peripheral blood collected from 62 emergency workers in 2018 were analysed in detail. In particular, one worker who was diagnosed with cancer and seven workers who had insufficient cells analysed (<1000 cell equivalents (CE)) were removed from analysis. In the remaining 54 workers, Poisson distribution was

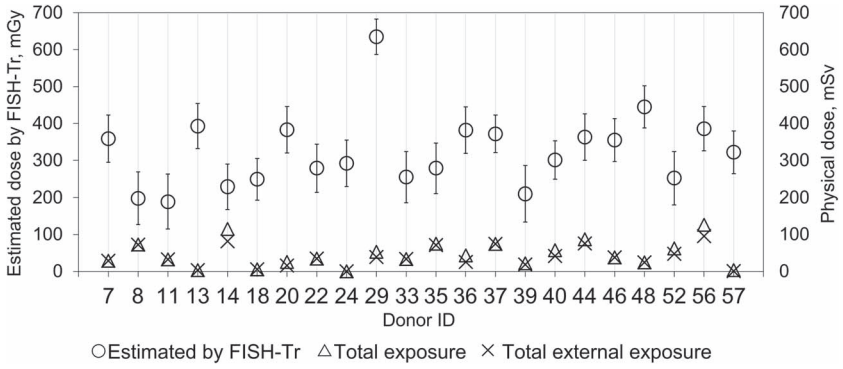

Figure 2. Comparison of reliable biological estimates and physical doses in 22 donors. The estimated doses of Donor IDs 7–57 were 359, 198, 189, 393, 229, 248, 383, 279, 292, 635, 255, 279, 382, 372, 210, 301, 363, 355, 445, 252, 386 and 322 mGy, respectively.

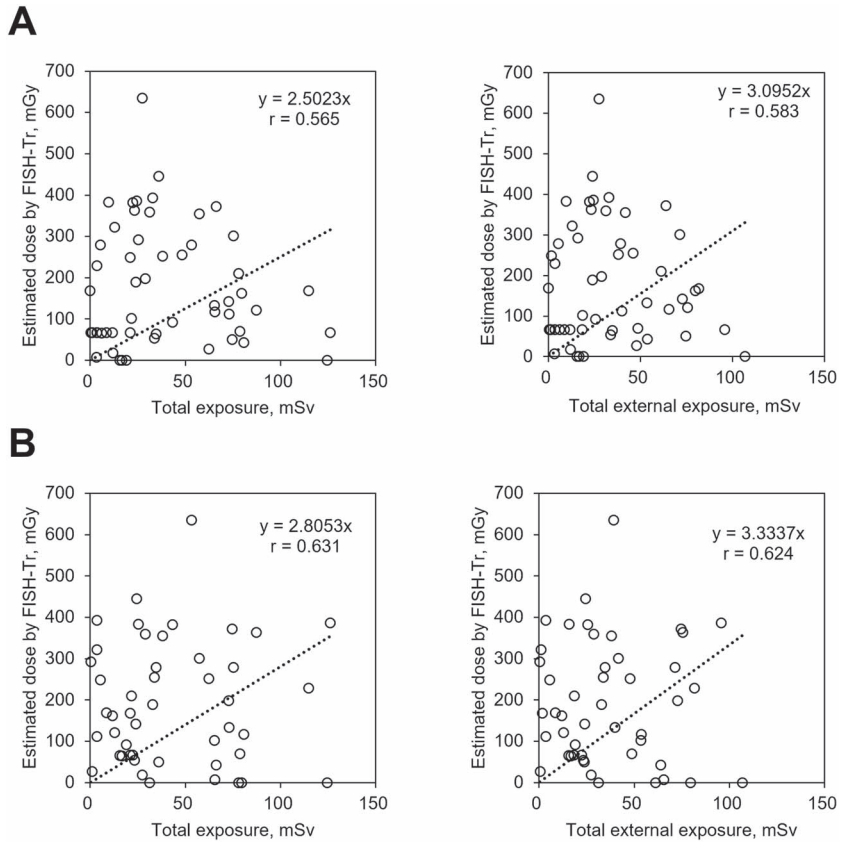

Figure 3. Correlation analysis between physical and biological dose assessments. (**A**) All emergency workers with more than 1000 CE analysed ($n = 54$), (**B**) emergency workers who had greater than zero Tr/CE after age-correction due to background Tr ($n = 47$).

evaluated as translocations induced by uniform whole-body radiation exposure follows a Poisson distribution. 33 out of 54 subjects deviated from the Poisson distribution. The range of estimated doses by FISH-Tr assay was 0–635 mGy and 22 workers showed reliable estimated doses of more than 189 mGy.

We then compared the biological estimated dose (mGy) calculated using FISH-Tr assay with the dose (mSv) calculated by physical dosimetry. In the 22 workers, biological doses were higher for all workers relative to total exposure and total external exposure up to the time of blood collection (Figure 2).

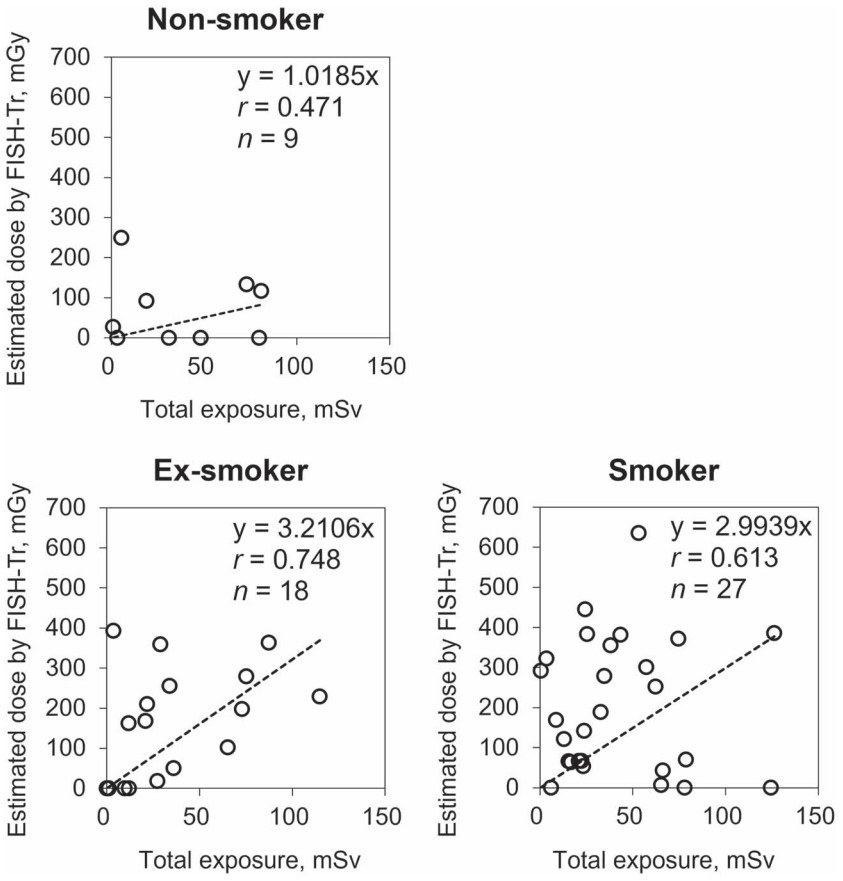

Figure 4. Stratified analysis of exposure dose correlation based on smoking habits. *P*-values of Non-smoker, Ex-smoker and Smoker were 0.90, 0.085 and 0.95, respectively.

Although the units of the estimated biological dose (mGy) and the physical dose (mSv) are different, the doses can be compared if we assume the same radiation dose is received uniformly over the entire whole body with a radiation weighting factor of 1. Here, the ratios of the estimated biological dose to the physical exposure dose are 80.19 ± 206.89 (emergency exposure), 48.23 ± 143.68 (total exposure), 113.1 ± 257.71 (emergency external dose) and 58.48 ± 152.12 times (total external dose).

Regarding the correlation between physical dose and biological dose, the estimated biological dose positively correlates with the total exposure dose ($r = 0.565$, $P = 0.65$) and the total external dose ($r = 0.583$, $P = 0.81$) (Figure 3A). In workers with more than zero Tr/CE after age-correction, there was also an increase in correlation coefficients with the total exposure ($r = 0.631$, $P = 0.63$) and the total external dose ($r = 0.624$, $P = 0.85$) (Figure 3B).

Effect of smoking on biological estimated dose

In our study, estimated biological doses correlated with physical doses but tended to be higher than recorded physical doses, which could be attributed to the workers' smoking habits. The smoking habits of the workers were divided into three groups: non-smokers, ex-smokers and smokers, and stratified analysis was performed. Workers with a smoking history, including ex-smokers, had a higher linear correlation coefficient between total exposure and estimated biological dose than workers without a smoking history. This reinforces that smoking is likely a contributing factor to the increased frequency of chromosome translocations, which subsequently increased the estimated biological dose (Figure 4).

This similar tendency was also observed for the Russian cleanup workers of the Chernobyl nuclear disaster in 1986[13]. In an international spontaneous

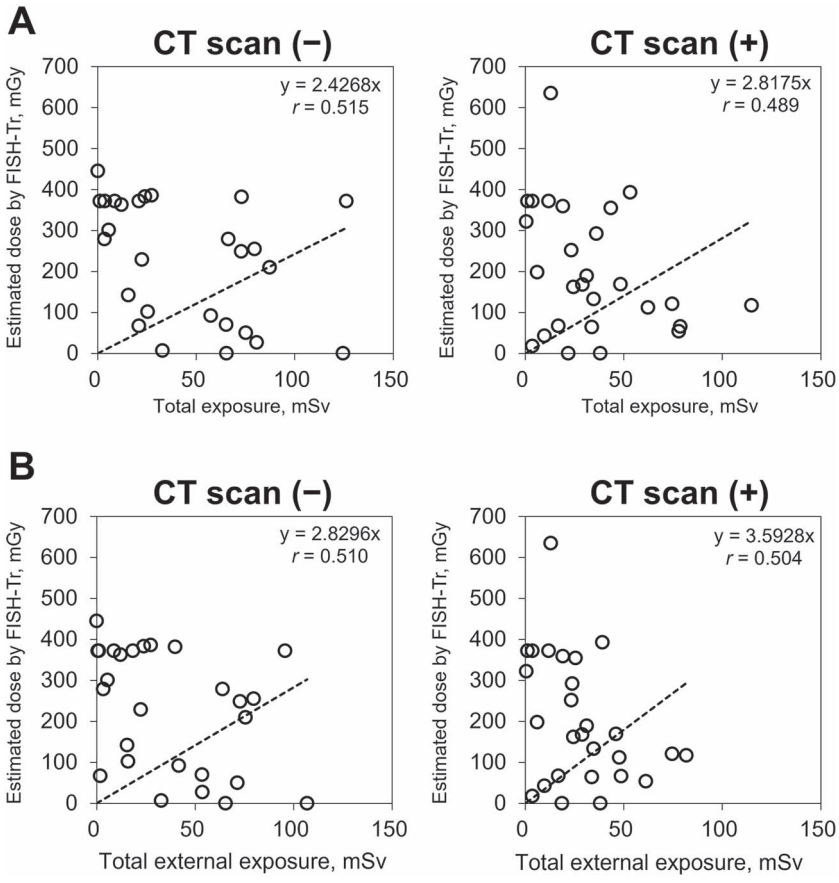

Figure 5. Stratified analysis of exposure dose correlation based on history of CT scan examinations. (**A**) Total exposure vs. estimated dose by FISH-Tr [*P*-values of CT scan (−), 0.04; CT scan (+), 0.10], (**B**) total external exposure vs. estimated dose by FISH-Tr [*P*-values of CT scan (−), 0.04; CT scan (+), 0.06].

translocation frequency survey, Sigurdson reported that smokers were 1.19 times more likely to have chromosome translocations than non-smokers[14]. As of now, no study has been performed evaluating the relationship between smoking and translocation frequency after chronic radiation exposure. Further analysis of the effects of the number of cigarettes smoked and secondhand smoke will be performed in the study to evaluate a possible smoking correction factor for more reliable biological dose estimates.

Effect of CT scan examination on biological estimated dose

In order to analyse the possible influence of medical exposure (i.e. CT scans) on the frequency of chromosome translocation, stratified analysis was performed based on the medical history of each worker. In the relationship between total exposure or total external exposure and estimated biological dose, there was no change in linear correlation in workers with or without a history of CT scan examinations (Figure 5). However, when analysing workers with a history of CT scan examinations only, the number of CT scan examinations and the dose difference between the estimated biological dose and the physical dose (cumulative exposure) showed a positive correlation (Figure 5).

From this, it was confirmed that the history of CT scan examination is a factor that influences biological dose evaluation. Furthermore, the number of CT scan examinations and the dose difference between the estimated biological dose and the physical dose (cumulative exposure) showed positive correlations (Figure 6). Removing donors with zero Tr/CE after age-correction showed a higher linear correlation coefficient (Figure 6B). The number of CT scan examinations could be causing the discrepancy between the estimated biological and physical doses.

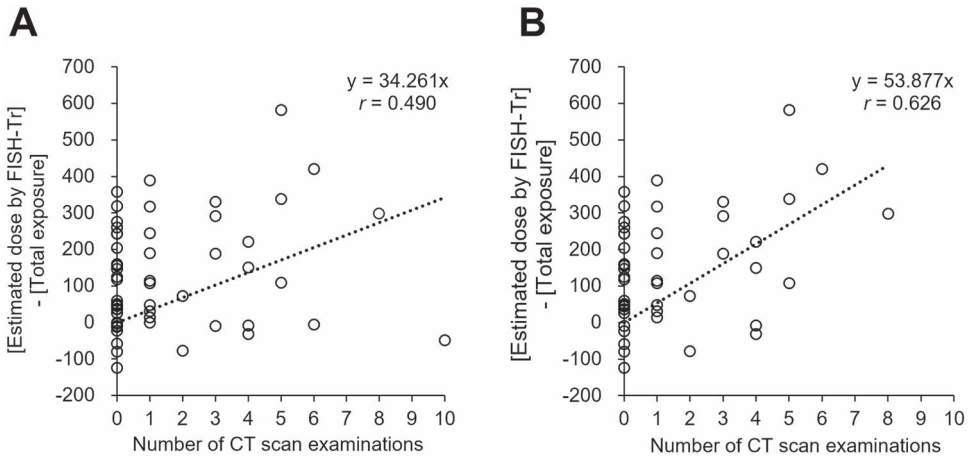

Figure 6. Comparison of the number of CT scans and dose difference between biological dose assessment and physical exposure. (**A**) Emergency workers with more than 1000 CE analysed ($n = 54$) ($P = 0.16$), (**B**) emergency workers who had greater than zero Tr/CE after age-correction due to background Tr ($n = 47$) ($P = 0.82$).

When analysing the CT scan history, information on the examination site (e.g. part of the body imaged) and the number of examinations could only be obtained. As the exposure dose from CT scan examination varies depending on the target organ, examination method and equipment used, it is difficult to calculate an accurate dose from medical exposure. In the future, the average dose from CT scan tests in Japan will be used to calculate the medical exposure and analyse the effect on biological dose estimation.

Conclusion

In 2018, peripheral blood from 62 workers who engaged in FDNPP emergency work in 2011 was collected for retrospective dose assessment using the FISH-Tr assay. The range of the estimated dose by FISH-Tr assay was 0–635 mGy in emergency workers and 22 workers had estimated doses of more than 189 mGy. Biologically estimated doses were higher in workers with physically measured total exposure recordings >70 mGy. Smoking and medical examination history are potential factors that could have caused higher estimated biological doses as the linear correlation was higher in smokers or for those who received prior CT scans. Thus, these factors likely contribute to the estimated dose discrepancies and need to be further studied to improve dose assessment strategies.

Funding

This work was supported by Industrial Disease Clinical Research Grants (Ministry of Health, Labour and Welfare, Japan).

Conflicts of interest

The authors declare no conflict of interests.

Acknowledgements

We thank Dr. Hiroyuki Yamaguchi and the staff members of Fukushima Prefecture Labor Health Center for their co-operation in peripheral blood collection from emergency workers.

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