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***Review***

# Could scoring tailed and dumbbell-shaped nuclei increase the sensitivity of micronucleus analysis as a biomarker of radiation exposure?

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**Abstract**

In the cytokinesis-block micronucleus (CBMN) assay, micronuclei (MNi), nucleoplasmic bridges (NPBs), and nuclear budding (NBUD) are the most commonly analysed morphological types of nuclear abnormalities. In contrast, tailed and dumbbell-shaped nucleus have historically received little attention in the CBMN assay. Interestingly, the incidence of tailed and dumbbell-shaped nuclei in lymphocytes is closely related with that of dicentric chromosomes or NPBs in the CBMN assay. To provide a better picture of the implications and significance of tailed and dumbbell-shaped nuclei as markers of radiation exposure, a literature review was performed in this study. Twenty articles were found in PubMed, PubMed Central, and manually searched. The articles were screened and those that met the inclusion criteria and did not meet the exclusion criteria were reviewed by all authors. At the end, nine articles were included. In conclusion, the assessment of *in vivo* tailed nuclei in blood smears and accounting for the occurrence of dumbbell-shaped nuclei in the CBMN assay can increase the sensitivity of the CBMN assay for biodosimetry involving a high dose exposure.

Keywords: Biomarker, dumbbell-shaped nuclei, micronuclei, radiation, tailed nuclei

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## Introduction

At the end of nineteenth century, William Howell and Justin Jolly identified micronuclei (MNi) in the blood cells of animals. These extra-nuclear bodies containing chromosomes or their fragments are also known as Howell–Jolly bodies, which are also observed in the erythrocytes of patients with severe anaemia (Luzhna et al. 2013, Hayashi 2016). In 1976, Countryman and Heddle developed a MNi assay for cultured human lymphocytes. Fenech and Morley then modified the technique using cytochalasin B and introduced an improved method: the cytokinesis-block micronucleus cytome (CBMN) assay, which has been widely used for genotoxicity assessment and human biomonitoring since then (Sommer et al. 2020). In the CBMN assay, a micronucleus (MN) is defined as a small extra-nuclear body that contains acentric chromosome fragments or chromosomes that failed to attach to mitotic spindles during mitosis and were excluded from the nucleus, resulting in MNi in daughter cells (Xiao et al. 2020). Since its creation, the CBMN assay has been one of the most common cytogenetic techniques for measuring the radiation exposure of biological systems. This measurement is known as biological dosimetry, or biodosimetry, which relies on biomarkers to estimate the level of radiation received by organisms (Sproull et al. 2017, Macaeva et al. 2018). In addition to MNi in binucleated cells (BNC), the CBMN assay scores DNA damage based on other biomarkers, such as nucleoplasmic bridges (NPBs), a marker of dicentric chromosomes, and nuclear buds (NBUDs), a marker of abnormal gene amplification. Not only lymphocytes can be analysed in the CBMN assay. Erythrocytes can also be used for biodosimetry using this technique. However, the upper limit of detection for red blood cells is 1 Gy and samples must be collected as soon as possible after radiation exposure, since ionizing radiation inhibits erythropoiesis (International Atomic Energy Agency 2011).

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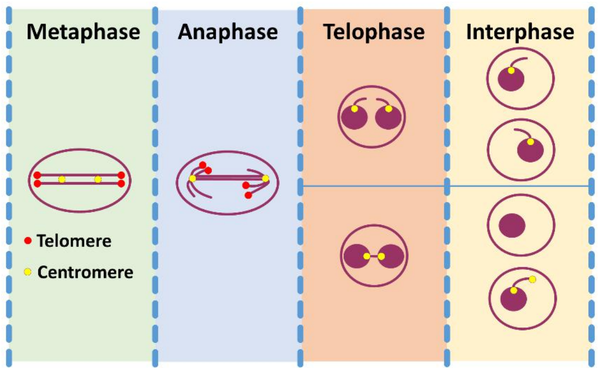


**Figure 1.** A simplified scheme illustrating the formation of tailed nuclei from dicentric chromosomes. For the complete scheme of tailed nuclei formation from dicentric and ring chromosomes refer to Kravtsov et al. (2014). This figure was re-drawn from Kravtsov et al. (2014).

Although MNi, NPBs, and NBUDs are the main morphological types of nuclear abnormalities, there are other abnormalities identified in the CBMN assay. These include tailed and dumbbell-shaped nuclei, which have historically received much less attention in the context of biodosimetry. Tailed nuclei in lymphocytes have been associated with dicentric chromosomes or NPBs in the CBMN assay. When the centromeres of dicentric chromosomes migrate to two opposite poles in cell during anaphase, a NPB is formed during telophase. Then, if the NPB undergoes a rupture during cytokinesis, it could result in the formation of tailed nuclei (Fig. 1) (Kravtsov et al. 2018). In 2014, Kravtsov et al. investigated the origin of tailed nuclei using telomere and centromere fluorescence in situ hybridization (FISH). The FISH analysis revealed that centromeres are mostly detected at the end of the tail, whereas telomeres are only found in the origin of the tail. These findings led to the conclusion that interphase tailed nuclei are due to the presence of dicentric chromosomes and chromosomal bridges with their subsequent breakdown. Thus, irradiated cells would go through a "breakage–fusion–bridge" cycle during mitosis, when would NPB break during cytokinesis and form tailed nuclei. In other words, tailed nuclei observed during interphase are produced by the breakdown of chromosomal bridges between dicentric chromosomes. Since the tip of the tail of tailed nuclei contains double-strand breaks (DSB), as identified by the DNA repair system, it can cross-link, form dicentric chromosomes, and generate new NPBs after anaphase (Kravtsov et al. 2014, Kravtsov et al. 2018).

Another morphological abnormality of nuclei that has been given little emphasis is the presence of dumbbell-shaped nuclei. Dumbbell-shaped nuclei were initially described in 1928 by Pelger as a nuclear abnormality of granulocytes in patients with tuberculosis (Constantino 2005). Interestingly, this form of abnormality is often detected in lymphocytes of Chernobyl accident liquidators but very rarely described. Dumbbell-shaped nuclei originate from the fusion of two nuclei, resembling the shape of a dumbbell or the number eight (Fig. 2). The formation of dumbbell-shaped nuclei differs from the nuclei bridging of NPBs, and it is associated with the formation of dicentric and ring chromosomes. Although the origin of dumbbell-shaped nuclei has been associated with the morphological characteristics of amitosis (direct cell division), in which the nuclear components are unequally distributed between the nuclei of daughter cells, the exact mechanism of the formation of this nuclear abnormality remains elusive. Still, Kravtsov and colleagues found that the structure of dumbbell-shaped nuclei is arranged by several NPBs that connects one nucleus to the other (Kravtsov et al. 2018). Moreover, they found that the number of cells containing dumbbell-shaped nuclei is a reliable predictor of ionizing radiation dose and correlates with the occurrence of chromosome abnormalities (Kravtsov et al. 2018). Thus, the frequency of lymphocytes bearing dumbbell-shaped nuclei is an attractive measure for biodosimetry testing, especially when one considers that counting of cells with dumbbell-shaped nuclei is much simpler than analysing metaphase chromosomes in cytogenetic assessments (Kravtsov et al. 2018). In fact, Kravtsov and colleagues recommended that biodosimetry testing of high dose exposures using the CBMN assay should include the analysis of dumbbell-shaped nuclei (Kravtsov et al. 2018).

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**Figure 2.** Illustration of dumbbell-shaped nuclei. This figure was re-drawn from Kravtsov et al. (2018)

Since analysing tailed and dumbbell-shaped nuclei is not currently recommended for biodosimetry testing or human biomonitoring studies using the CBMN assay, in this work, we reviewed all studies that evaluated these two nuclear abnormalities in lymphocytes of individuals which experienced a radiation exposure during their life. In addition, studies that used tailed nuclei as specific biomarker of radiation exposure in non-human such as fish also included in this review to obtain more comprehensive understanding on how this two markers might be included in CBMN assay for biodosimetry.

## Methods

### Search strategy

Two digital databases (PubMed and PubMed Central/PMC) were used to search for articles published from 1978 to 2021. The following medical subject headings (MeSH) were used as search entries: "Tailed", "Nuclei", "Radiation", and "Dumbbell" (Table 1). To be included in this review, the articles found in the databases had to meet following inclusion criteria: to be written in English or have sufficient information in the English version of the abstract; to have assessed individuals or organisms who had been exposed to radiation or blood samples which were exposed to radiation *in vitro*; to have evaluated tailed and/or dumbbell nuclei in lymphocytes or addressed the frequencies of tailed and/or dumbbell nuclei. Review articles were also included to obtain a comprehensive picture about the relevance of tailed and dumbbell nuclei. Articles were excluded if the full-text was unavailable. Lastly, the keywords "tailed", "dumbbell", "nuclei", "radiation", and "lymphocytes" were also used in manual search for publications.

### Data extraction

The following information was retrieved from the articles that met the inclusion criteria and did not meet the exclusion criteria: author information; population used in the study; type of cells where tailed and dumbbell-shaped nuclei was analysed (lymphocytes in blood smears or BNC in the CBMN assay); and study outcomes

**Table 1.** Databases and keywords used to search for articles

| Database/Source | MeSH/Terms | Results |
|---|---|---|
| PubMed | ((Tailed[Title/Abstract]) AND (Nuclei[Title/Abstract])) AND (Radiation[Title/Abstract]) | 10 articles |
| PMC | (((Tailed[Abstract]) AND Nuclei[Abstract]) AND Dumbbell[Abstract]) AND | 1 article |
| PMC | ((Tailed[Abstract]) AND Nuclei[Abstract]) AND Radiation[Abstract] | 4 articles |
| Manual | tailed, dumbbell, nuclei, radiation, lymphocytes | 9 articles |

**Table 2.** Characteristics of the studies that met the inclusion criteria

| Author, Year, Title | Subjects/Organisms | Type of analysis (blood smear or *in vitro* human lymphocyte culture) | Results | Reference |
|---|---|---|---|---|
| Kravtsov et al., 1997, "Tailed nuclei are a possible cell marker of radiational effects" | 135 male Chernobyl liquidators. The control group consisted of 50 healthy males | Blood smear | The mean frequency of lymphocytes containing tailed nuclei in the liquidators group was 0.59%, whereas that in the control group was 0.15%. The difference was statistically significant ($p<0.001$) | (Kravtsov *et al.* 1997a) |
| Kravtsov et al., 1997, "Morphological anomalies in "tailed" lymphocyte nuclei and their connection with dicentric chromosomes in irradiated patients" | 100 male Chernobyl liquidators and 1 patient that had been exposed to radiation in an atomic submarine accident. The control group consisted of 50 healthy males | Blood smear | Several characteristics of tailed nuclei were described as follows. First, tailed nuclei usually have a narrow outgrowth protruding to the cytoplasm and frequently possesses a terminal extension. The length of the tail ranges from 2 to 7 µm and have a similar colour and intensity compared to the nucleus. The mean frequency of lymphocytes containing tailed nuclei in the liquidators group was 0.43%, whereas that in the control group was 0.15% | (Kravtsov *et al.* 1997b) |
| Kravtsov et al., 2000, "Tailed nuclei and dicentric chromosomes in irradiated subjects" | 203 male Chernobyl liquidators and 4 other males exposed to radiation under different circumstances. One had his blood collected 6 years after being accidentally irradiated with 0.6 Gy. Another had his blood collected 6 months after being irradiated with up to 20 Gy on the skin. Another had his blood collected 3 days after having his body partially irradiated due to radiotherapy treatment with a total dose of 6 Gy. Another had his blood collected 7 years after being accidentally irradiated with about 1.5 Gy. The control group consisted of 114 healthy males | Blood smear | Authors identified and described 16 types of tailed nuclei (Figure 4). Types 6–8 were those predominantly observed in the control group (Figure 5). The mean frequency of tailed nuclei in the irradiated subjects was 0.50%, whereas that in the control group was 0.14%. The highest frequency of tailed nuclei in irradiated subjects was 3.2%. This value is much higher than the highest frequency of tailed nuclei found in Chernobyl liquidators, which was only 0.6% | (Kravtsov *et al.* 2000) |
| Kravtsov et al., 2014, "Lymphocytes with "Tailed" Nuclei (LTN) in Blood Smears as the Easiest Biomarker of Radiation Exposure That is Acceptable in Emergencies" | Similar to Kravtsov et al. (2000). | Blood smear | Centromere sequences were detected predominantly in the base and/or in the chromatin enlargement region of the tip of the "tails", whereas telomere sequences were only detected in the latter region. The authors recommended that the tailed nuclei analysis could be used as a convenient initial testing in large radiation accidents and provide results faster than other analyses. The authors also underscored that the *in vivo* tailed nuclei analysis is much simpler and cheaper than other analyses to be used in emergencies | (Kravtsov *et al.* 2014) |
| Kravtsov et al., 2017, "Nuclear Abnormalities of Lymphocytes as the Simplest Markers for Bioindication Test in Case of Mass Casualty | Review article | None | These two articles described in detail all types of lymphocyte nuclear abnormalities in blood smears. They also highlighted the importance of evaluating tailed and dumbbell-shaped nuclei in emergency situations due to the simplicity of the procedure, which can be conducted in any | (Kravtsov *et al.* 2017) |

| | | | | |
|---|---|---|---|---|
| Events Involving Radiation Exposure" | | | biodosimetry laboratory. In addition, the authors indicated that tailed and dumbbell-shaped nuclei are specific biomarkers for environmental risk analyses of organisms, including humans, exposed to radiation intentionally or accidentally | |
| Anbuman et al., 2017, "Various types of nuclei pathology in somatic cells as universal indicator of ionizing radiation exposure" | Review article | None | | (Anbuman *et al.* 2017) |
| Kravtsov et al., 2018, "The frequency of lymphocytes containing dumbbell-shaped nuclei depends on ionizing radiation dose and correlates with appearance of chromosomal aberrations" | Peripheral blood from a single donor (female, 30 years old) without history of smoking and drinking | Lymphocytes exposed to 0.1–2.0 Gy *in vitro* | The occurrence of tailed nuclei positively correlated with the frequency of dicentric and ring chromosomes. Dicentric and ring chromosomes led to the formation of other nuclear abnormalities and not only tailed nuclei. There was a very strong correlation between the frequency of dumbbell-shaped nuclei and the frequency of dicentric and ring chromosomes. Most of dicentric chromosomes are formed from dumbbell-shaped nuclei. The occurrence of dumbbell-shaped nuclei should receive more attention in biodosimetry using the CBMN assay. The biodosimetry using the CBMN assay needs a significant correction for the occurrence of dumbbell-shaped nuclei in samples irradiated at high doses | (Kravtsov *et al.* 2018) |
| Anbumani & Mohankumar, 2012, "Gamma radiation induced micronuclei and erythrocyte cellular abnormalities in the fish Catla catla" | Freshwater fish *Catla catla* (Hamilton, Family: Cyprinidae) exposed for 42 h at a dose rate of 0.002 Gy/min to a total dose of 5 Gy and at a dose rate of 3.2 Gy/min to a total dose of 5 Gy. Blood was obtained at five fish each duration (days 3, 6, 12, 18, 30, 45, 90, 135 and 202) by cutting the caudal peduncle and immediately processed for EMNCA. | Blood smear | The tail nuclei were shown in fish exposed to acute gamma radiation between days 12 and 30, with the highest frequency on day 18. | (Anbumani & Mohankumar 2012) |
| Anbumani & Mohankumar, 2015, "Nucleoplasmic bridges and tailed nuclei are signatures of radiation exposure in Oreochromis mossambicus using erythrocyte micronucleus cytome assay (EMNCA)" | Freshwater fish *Oreochromis mossambicus* (Peters, Family: Cichlidae) exposed of 2 mGy/min to a dose of 2.5, 5, and 10 Gy. Blood samples were obtained from the caudal vein at various time intervals (days 3, 6, 12, 18, and 30) and directly processed for the EMNCA. | Blood smear | Nucleoplasmic bridges and tailed nuclei increased significantly between days 3 and 12 in fish exposed to 5 and 10 Gy. Fish exposed to 5 Gy showed a substantial rise from day 3 forward, but 10 Gy exposed animals showed an increase from day 12 onward, with significance lasting until day 30 post-exposure. Nucleoplasmic bridges and tailed nuclei also were shown to have significant correlation ($p<0.05$). | (Anbumani & Mohankumar 2015) |

## Results and discussion

We retrieved 24 articles from PubMed, PMC and manual search using two or more combinations of keywords. Ten articles that did not correlate the use of tailed and dumbbell-shaped nuclei with radiation exposure were excluded. Other articles that were duplicated were excluded. At the end, nine articles retrieved from PubMed, PMC, and manual search were included in this review (Fig. 3). The information retrieved from each study is presented in Table 2.

### Tailed nuclei in blood smears as biomarkers of radiation exposure

Most of the studies included in our review evaluated tailed nuclei in blood smears. Kravtsov et al. evaluated 135 male Chernobyl liquidators that were exposed to radiation 6–11 years before blood collection. The control group comprised 50 healthy males. The evaluation of 500 lymphocytes in blood smears from each donor was performed in this study. The authors also assessed the frequency of dicentric chromosomes in 47 subjects of the liquidators group. This study revealed that more than 85% of the liquidators had tailed nuclei in their lymphocytes. In the control group, more than 50% of the subjects had no tailed nuclei in their lymphocytes. The mean incidence of lymphocytes containing tailed nuclei in the liquidators group was 0.59%, whereas that in the control group was 0.15%. The statistical analysis showed that the difference was statistically significant ($p$<0.001). The authors also found a quite strong positive correlation between frequency of "*in vivo* lymphocytes with tailed nuclei" and "frequency of *in vitro* culture lymphocytes with dicentrics". The correlation was also statistically significant ($p$<0.001), with a correlation coefficient value of 0.73. In this article, Kravtsov and colleagues also published images of each tailed nucleus type they found, which were further described in detail in another publication (Kravtsov *et al.* 1997a)

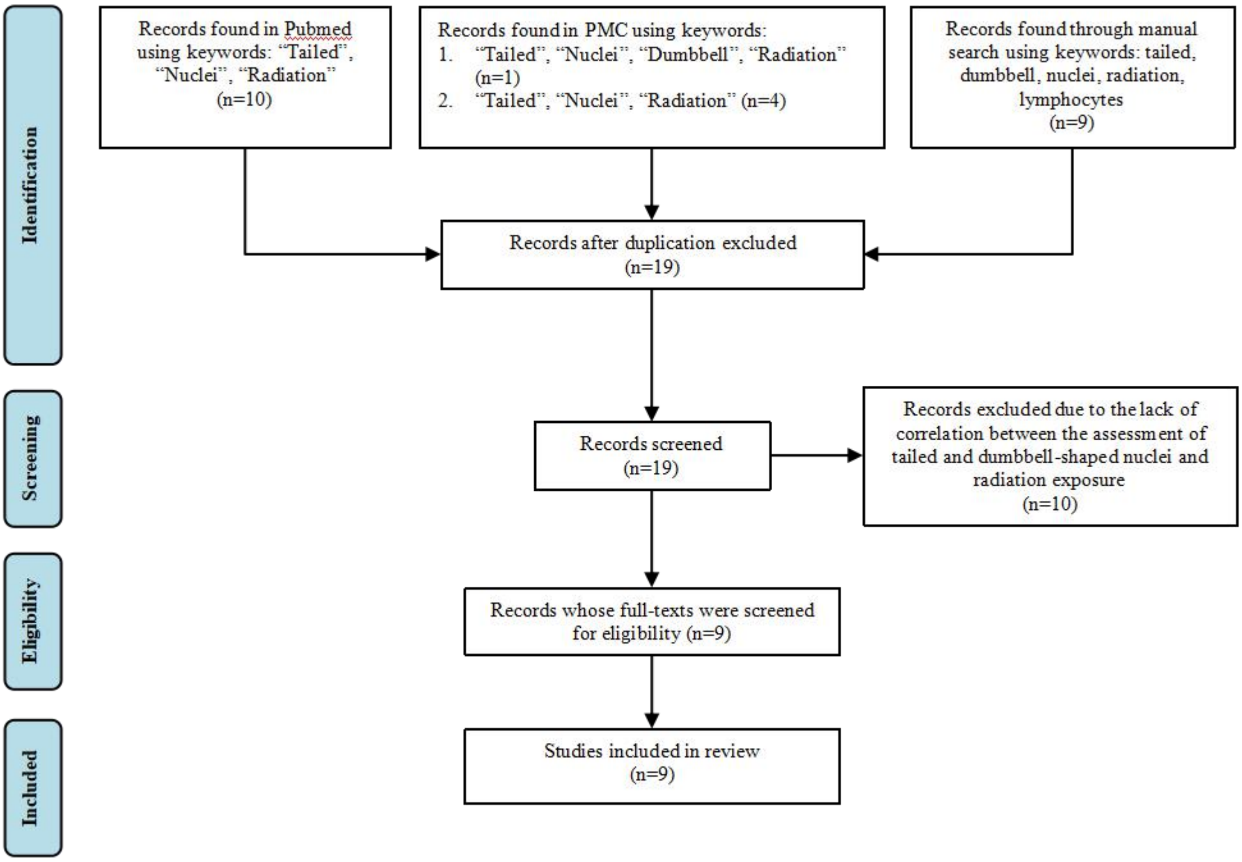


**Figure 3.** Flow diagram of the selection of articles for this review.

In the same year, Kravtsov and colleagues also published an article covering samples from 100 male Chernobyl liquidators and one man that was accidentally exposed to radiation in an atomic submarine (Kravtsov et al. 1997b). Fifty healthy males served as the control group. In this study, the authors described several characteristics of tailed nuclei, including a narrow outgrowth protruding to the cytoplasm and a terminal extension. The length of the tails ranged from 2 to 7 µm, and tails were similar to the nucleus in terms of colour and intensity. The mean frequency of lymphocytes containing tailed nuclei in the liquidators group was 0.43%, whereas that in the control groups was 0.15%. Similar to their previous study, the authors evaluated the correlation between "*in vivo* lymphocytes with tailed nuclei" and "frequency of *in vitro* culture lymphocytes with dicentrics". A total of 27 donors from the liquidators group were assessed for the frequency of dicentric chromosomes, and a significant correlation with a coefficient value (r) of 0.74 was found.

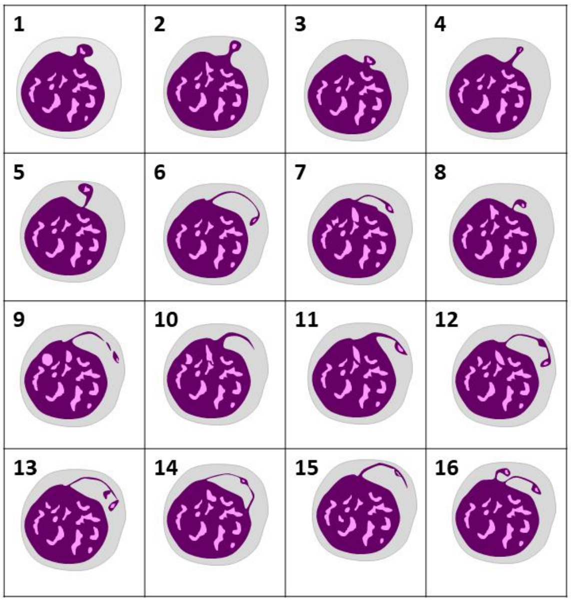


**Figure 4.** The 16 types of tailed nucleus identified in human lymphocytes. This figure was re-drawn from Kravtsov et al. (2017).

Three years later, in 2000, Kravtsov and colleagues comprehensively described all types of tailed nucleus found in blood smears. This time, Kravtsov and his team evaluated 203 male Chernobyl liquidators and 4 other males exposed to radiation under different circumstances. One had his blood collected 6 years after being accidentally irradiated with 0.6 Gy. Another had his blood collected 6 months after being accidentally irradiated with up to 20 Gy on the skin. Another had his blood collected 3 days after having his body partially irradiated due to radiotherapy treatment with a total dose of 6 Gy. Another had his blood collected 7 years after being accidentally irradiated with about 1.5 Gy. The control group consisted of 114 males (19–60 years old), 68 females (19–60 years old), and 37 children (3–6 years old). Sixteen types of tailed nuclei were identified (Fig. 4) and described in detail in the publication. The results of this study showed that the tailed nucleus types predominantly observed in the control group are the types 6–8 (Fig. 5). The mean frequency of tailed nuclei in irradiated subjects was 0.50%, whereas that in the control men was 0.14%. Noteworthy, the highest frequency of tailed nuclei in irradiated subjects was 3.2%. This value is much higher than the highest frequency of tailed nuclei frequency recorded for the Chernobyl liquidators, which was only 0.6% (Fig. 6) (Kravtsov et al. 2000).

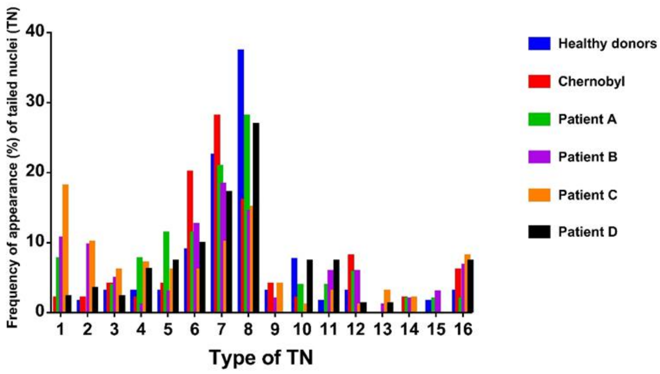


**Figure 5.** Frequencies of the 16 different morphological types of tailed nucleus (TN) found by Kravtsov et al. (2000). This figure was drawn based on the data in Kravtsov et al. (2000).

In 2014, Kravtsov and his team published an article that was quite similar to their previous publication in 2000. However, this time, they performed fluorescence in situ hybridization (FISH) using two probes to hybridize with centromere and telomere heterochromatin in the tailed nuclei. The most interesting finding of this study is that centromeres were predominantly located in the base and/or in the chromatin enlargement region at the tip of the tails, whereas telomeres were detected only in the latter region. The authors also did not find any tail containing more than two centromere signals and one telomere signal. This finding indicates that tailed nuclei might be formed by one and/or two chromosomes. Moreover, based on the data on centromere and telomere regions in the tailed nuclei, one cannot exclude the possibility of nuclear tails containing the chromatin of an entire dicentric chromosome instead of only a half of the broken dicentric chromosome. Thus, the authors chose to refer to these nuclear abnormalities as "tailed nuclei" instead of "semi-bridge". The authors also emphasized that the incidence of lymphocytes containing internuclear chromatin bridges must be considered, when performing the tailed nuclei analysis. Bridges, are a much rarer phenomena than tails, and the incidence of tailed nuclei is significantly higher than that of bridges in circulating lymphocytes. Lastly, the authors also emphasized that the *in vivo* tailed nuclei analysis in blood smears is not an alternative to the classic *in vitro* radiation markers, such as dicentric or micronuclei in the CBMN assay. Rather than an alternative, the tailed nuclei analysis might become an initial test for large radiation accidents (involving hundreds or thousands of people) and provide results faster than other analyses. Another advantage is that the *in vivo* tailed nuclei analysis can be conducted using fingertip blood smears, which are much simple and cheap to be used in emergencies (Kravtsov et al. 2014).

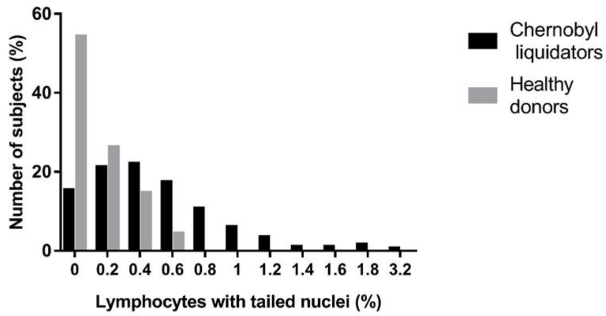


**Figure 6.** Frequencies of peripheral blood lymphocytes with tailed nuclei found in Chernobyl liquidators and male control subjects. This figure was re-drawn from Kravtsov et al. (2000).

The two review articles included in this review were published by Kravtsov et al. (2017) and Anbuman et al. (2017). These two articles explained in detail all types of nuclear abnormalities found in blood smear lymphocytes, including MNi, NPBs, tailed, and dumbbell-shaped nuclei. Overall, these two articles highlight the importance of these nuclear abnormalities during emergencies due to the feasibility of analysing them in virtually any biodosimetry laboratory. Moreover, these nuclear abnormalities are also considered to be specific for environmental risk analysis of organisms, including humans, exposed to radiation intentionally or unintentionally.

**Tailed and dumbbell-shaped nuclei analysis in the CBMN assay**

From the articles found using our search strategy, only one experimentally assessed tailed and dumbbell-shaped nuclei in BNC (Kravtsov et al. 2018). In that study, peripheral blood samples were collected from a single donor (female, 30 years old, and without history of smoking or drinking), and irradiated with nine different doses: 0.0, 0.1, 0.2, 0.4, 0.5, 0.7, 1.0, 1.5, and 2.0 Gy. The authors aimed to evaluate the interplay between NPB and the incidence of tailed and dumbbell-shaped nuclei in human lymphocytes cultured using the CBMN assay and exposed to X-ray radiation at doses from 0 to 2

Gy *in vitro*. Overall, the study confirmed that the origin of tailed nuclei is the breakage of NPBs. The authors also found that the frequency of tailed nuclei positively correlated with that of dicentric and ring chromosomes (r = 0.89, p<0.005). Dicentric and ring chromosomes led to the formation of other nuclear abnormalities and not only the tailed nuclei. Indeed, the frequency of dicentric and ring chromosomes increased sharply from 0.7 Gy on, whereas that of tailed nuclei did not increase proportionally (Fig. 7). On the other hand, a very strong correlation was found between the frequency of dumbbell-shaped nuclei and the frequency of dicentric and ring chromosomes (r = 0.99, p<0.05). The frequencies of dumbbell-shaped nuclei in samples exposed to 1.5 and 2.0 Gy were significantly higher than those in samples exposed to the lower doses. Interestingly, a similar trend was also observed for the frequency of chromosomal aberrations. Based on this finding, the authors concluded that most of dicentric chromosomes formed as dumbbell-shaped nuclei at high radiation doses. Thus, the dumbbell-shaped nuclei should receive more attention in biodosimetry using the CBMN assay. The authors also suggested that biodosimetry using the CBMN assay requires a significant compensation for the occurrence dumbbell-shaped nuclei in samples irradiated with high doses of radiation.

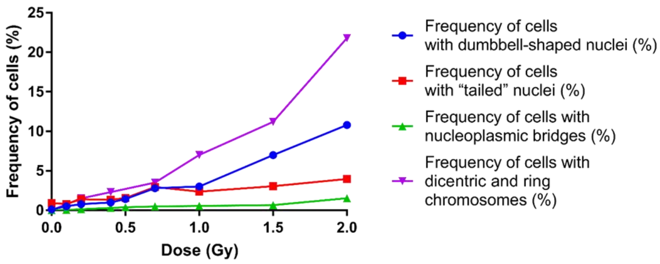


**Figure 7.** Frequencies of peripheral blood lymphocytes with different nuclei abnormalities after exposure to X-ray (0.1–2.0 Gy). This figure was re-drawn from Kravtsov et al. (2018).

## Tailed and dumbbell-shaped nuclei analysis in human biomonitoring

To date the analysis of tailed and dumbbell-shaped nuclei in human biomonitoring, particularly for individuals living at high background radiation area (HBRA), is very rarely conducted. This contrasts with the fact that tailed nuclei are observed not only in human lymphocytes but also in erythrocytes of embryonic fish exposed to radiation *in vivo* (Braham et al. 2017, Khetsuriani et al. 2019, Khatun et al. 2021). For this reason, the "erythrocyte micronucleus cytome assay" (ECMNA) has been used in aquatic cytogenotoxicity (Anbumani & Mohankumar 2011). Previous preliminary studies by our research group revealed that the frequency of MNi in people living in Botteng Village (Mamuju, Indonesia) was not statistically different from that in the control group (Ramadhani et al. 2018). Botteng Village is classified as a HBRA due to the annual effective dose received by its inhabitants, which reaches up to 27 mSv, based on the median values of external and internal exposures (Hosoda et al. 2021). Although the ventilation of typical houses in Botteng Village is considered to be adequate for maximal gas exchange, the mean radon concentration indoors in Botteng Village might reach 398 Bq/m3. Additional evaluation using the tailed and dumbbell-shaped nuclei markers in the BNC of Botteng Village inhabitants, as well as the *in vivo* analysis of tailed and dumbbell-shaped nucleus in the blood smears, could provide valuable information on the actual cytogenetic and health status of the population. Figure 8 shows a proposed scheme to monitor the population of the Botteng Village using the CBMN assay. The tailed and dumbbell-shaped nuclei might also have their frequencies considered in the in the G0 MN assay. In our previous study, we already used the G0 MN assay to evaluate the radioadaptive response (RAR) in lymphocytes of Botteng Village inhabitants (Ramadhani et al. 2018). The G0 MN assay that we performed in our previous study mainly relied on the evaluation of MNi in BNC. Thus, the use of other nucleus abnormalities biomarkers, such as tailed or dumbbell-shaped nuclei, in the G0 MN assay might provide addition information. In that regard, Dr. Viacheslav Kravtsov, the main author of most of the articles included in this review, believes that the analysis of dumbbell-shaped nuclei might give a more reliable result than that of tailed nuclei since this maker is more relevant and easier to recognise than tailed nuclei (Viacheslav Kravtsov, personal communication, April 21, 2021). Moreover, although a comprehensive description of tailed nuclei morphometry is available, (Kravtsov et al. 2000, Kravtsov et al. 2014, Kravtsov et al. 2017), it might be difficult to distinguish tailed nuclei from nuclear buds in BNC.

## Tailed nuclei analysis in fish

The MN assay has recently gained popularity in aquatic genotoxic study due to its ease of application to fish and other aquatic animals (Anbumani & Mohankumar 2019). However, ionizing radiation-induced DNA damage in fish has received little attention, with only a few investigations utilizing the EMNCA in fish exposed to ionizing radiation. In addition to MN, various nuclear abnormalities in piscine erythrocytes have been investigated as prospective indications of genotoxicity in recent years (Anbumani & Mohankumar 2012). In the Anbumani and Mohankumar study (2012) that evaluated MN and other erythrocyte nuclear abnormalities in fish, Catla catla (Ham.) exposed to acute and protracted gamma radiation exposures, the authors found that tailed nuclei were observed between days 12 and 30 with the frequency peaking on day 18 (Fig. 9). It is possible that the tailed nuclei seen in their work are in fact broken bridges, since nuclear buds have been demonstrated to occur when a nucleoplasmic bridge between nuclei breaks and the remains shrink back toward the nuclei. Another study by Anbumani and Mohankumar in 2015 that used Tilapia fish (Oreochromis mossambicusi) which exposed to dose of 2.5, 5, and 10 Gy (dose rate of 2 mGy/min) showed the significant increase of nucleoplasmic bridges and tailed nuclei between days 3 and 12 in fish exposed to 5 and 10 Gy. The authors stated that the tailed nuclei can be used as unique cytogenetic markers of low-dose gamma radiation in fish.

## Conclusion

Overall it can be concluded that the assessment of *in vivo* tailed nuclei in blood smears together with the dumbbell-shaped nuclei analysis in the CBMN assay might increase the sensitivity of the CBMN assay for biodosimetry and human biomonitoring purposes.

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