THE TRANSMISSIBILITY OF STABLE ABERRATIONS

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ABSTRACT:
An important requirement for using chromosomal aberration analysis for biological dosimetry is that the aberration yield, as observed in blood T-lymphocytes has not decreased significantly between the time of irradiation and blood sampling. The most common using chromosomal aberration for biological dosimetry is dicentrics are referred to as unstable aberrations in the sense that their frequencies decline with time and so the biological indicator of damage declines. The half-life of this loss is sometimes suggested to be about 3 years, but there is clear evidence that decrease in dicentric yield is much more rapid after a high dose.

The translocations are referred as stable aberrations however have a greater likelihood of successfully negotiating mitosis and passing into viable daughter cells. It is for this reason that translocations are recommended as the aberration of choice for retrospective biological dosimetry in situations where irradiation has been protracted over long periods or a long time has elapsed before blood sampling. Although the translocations are accepted as stable aberrations, it has been showed that the translocation frequency decreases period of time.

In this paper, in order to understand the suitability of the translocations for using retrospective studies, the results of the different laboratories on the transmissibility of the stable type of radiation-induced aberrations in lymphocytes has been summarized.

INTRODUCTION
In the case of overexposure, it is important to estimate dose absorbed by exposed persons immediately after exposure in order to plan their therapy and at longer times after exposure to assess possible health consequences. In some situations, the victim has not been wearing a physical dosimeter during the accident. Even if the physical measurement of dose is available, an independent dose estimation by biological methods can be very useful. The measurement of absorbed dose using biological methods called as biological dosimetry.

There are some requirements to use a biological material as biological dosimetry:
♦ Sampling should be easy, and the taken sample should represent whole body
♦ To have a synchronize population
♦ To available establishing in-vitro dose response curve
♦ To have low level aberration background, and variability between individuals should be small.
♦ To have stability with time post exposure,
Unstable chromosomal aberrations, particularly dicentrics, in human peripheral blood T-lymphocytes have been used for estimation of the absorbed dose by individuals for many years. Dicentrics’ analysis in peripheral blood T-lymphocytes is accepted as the most sensitive biological dosimetry method of measuring radiation exposure. It is very easy to take blood sample and a few ml blood sample is enough to do analysis. Because of the regular lymphocyte circulation, taken sample represent whole body. More than 90% of T lymphocytes are in G0 phase therefore it has synchronize population. The calibration curves for dicentrics which obtained using human lymphocytes exposed in vitro, have been shown to be identical to those exposed in vivo (1). Because of the background level is 1 dicentric in 2000 metaphase cells, 0.1 Sv of low LET radiation and 0.01 Sv for fission spectrum neutrons could be estimated using dicentric analysis (2).

Dicentric analysis has been successful on dose estimation at much serious radiation accident such as Goiana, and Chernobyl. It is currently the most fully developed technique that could be used for dose estimation in the case of over exposure. However the system using dicentrics unfortunately has one disadvantage that their frequencies decline with time. Although the half-life of their loss, suggested to be about 3 years, because of the T-lymphocytes have 3 years half life in peripheral blood, there is clear evidence that decrease in dicentric yield is much more rapid after a high dose. Many studies showed that the half life of dicentric is changing between 5 months and 36 months depend on the absorbed dose. (3,4) For this reason, dicentric analysis is not effective when a dosimetry is required for chronic or multiple exposures over a long period of time.

The stable aberrations, particularly translocations however suffers very much less loss with time and persist many years. Measurement of the low translocation frequencies has been difficult and time consuming using conventional staining or banding analysis. However, the recent developed technique, called as Fluorescence in situ hybridization (FISH) with chromosome specific composite DNA probes (i.e. chromosome painting) allows analysis of translocations, and more convenient to use routinely than conventional techniques. Painting is particularly useful for detecting structural chromosome rearrangements.

**METHODOLOGY**

The FISH technique with chromosome specific DNA probes provides uniform labelling of the homologues of a particular target chromosome along their entire length. Because of the procedure is time and money consuming, particular chromosome pairs are painted instead of all pairs of chromosomes. Generally 3 chromosomes are selected. Painting probes in a cocktail of three chromosomes cover approximately 20% of the total genomic DNA content. In addition, some laboratories are included a centromeric probe for visualisation of centromere in order to prevent misclassification of aberrations. The formulas which are proposed by Lucas et al (5,6) could be used for comparison of data obtained with different chromosome cocktails. This
formulas are based on the assumption that the probability of a chromosome being involved in a particular exchange aberration is proportional to its DNA content.

Two types of translocations are used for dosimetrical purpose. Reciprocal (or complete) translocation and Terminal (or incomplete) translocations. (Figure 1, 2)

**Figure 1:** Reciprocal translocation (RT)

**Figure 2:** A complex rearrangement

It has been shown that dicentrics and reciprocal translocations are induced at equal frequency (7).

**STUDIES ON PERSISTENCE OF TRANSLOCATION**

There are many studies performed by different laboratories to understand which kind of translocation is more persist than the other, or what is the chance of transmissibility of these translocations to next cell division. These studies could be classified as

- a) in vivo studies
- b) in-vitro studies.
In-vivo studies:

In-vivo studies could be divided into two categories as animal studies, and the cases of overexposure.

The stability of radiation induced translocation frequencies were examined using animals by many researchers.

Two different Rhesus monkey study showed that total translocation yield is more persistent than dicentrics. Darroudi et al (8) irradiated Rhesus monkey with 5 Gy X-ray as whole body. In this study, blood samples were taken 52 h, 2 years, and 5 years after the exposure and total translocation yields were compared. No significant decrease were observed on genomic equivalent total translocation yield between 52 h and 2 years. However the translocation yields of 5 years after exposure is dropped to about 50%. Lucas et al (9) measured translocation frequencies in six exposed rhesus monkeys to high energy protons by NASA in 1965. They reported that the stability of the translocation frequency following whole-body exposures at 0.56-2.25 Gy of rhesus monkey is 28 years which is equivalent to about 90 years in human.

According to another study which is established to measure the persistence of radiation induced translocations, reciprocal translocations are more stable than non-reciprocal translocations. In this study female mice were exposed as whole body 0,1,2,3 and 4 Gy Cs-137 gamma rays (10). Bone marrow and peripheral blood cells metaphase chromosomes were examined for each group 1,8,15 and 30 days post irradiation. The percentage of reciprocal translocations did not significantly decreased with time either bone marrow or peripheral blood cells at least doses of 3 Gy or lower while non-reciprocal translocations induced by doses of 3 Gy or lower remained unchanged in the peripheral blood, but decreased after a week in the bone marrow, then remain constant. At the same study dicentrics decreased with time in both tissues, almost none of them remained at the end of 8 days.

In addition to in-vivo animal studies, dose estimation with using FISH analysis in the case of overexposures were also performed by many researcher. Atomic bomb survivors, Goiana and Chernobyl accident and also occupationally overexposure cases give a chance to understand the suitability of FISH analysis for dosimmetrical purpose.

In order to discuss the availability of using translocation frequency for dose estimation many years, even decades after whole-body exposure, Lucas et al. examined 20 Hiroshima atomic-bomb survivors blood samples, 45 years after their exposures (5). In this study they observed that, individual translocation frequencies of 20 subjects dose estimation were very close to doses that expected from in-vitro dose-response curve.

FISH analysis was also performed on Techa River population and Mayak workers almost 40 years after the Mayak nuclear accident. At the end of 1940’s and early 1950’s a radiation accident occurred due to release of radioactive waste from Mayak Nuclear industrial complex.
into Techa River, Southern Urals. The workers doses were changing between 326 mSv for reactor workers and annual 700 mSv (external gamma and plutonium aerosol). 73 possibly exposed resident along the Techa River and 75 workers of the Mayak nuclear industrial complex were examined by using FISH analysis (11,12). A total 39 healthy person were served as control. Depend on their radiation exposure history, higher translocation yield were observed at Techa river population than control group. For Mayak workers, dicentric frequency and translocation frequency for plutonium incorporated group were not significantly different than control group, however, the pooled data set showed a significant linear dependence of the translocation frequencies on cumulative external gamma doses. Individual biodosimetry estimates, ranging between 0.5-1.8 Gy.

Many researchers estimated absorbed dose by heavily irradiated employees of Chernobyl reactor (13) and populations living in contaminated areas in Gomel and Estonian Chernobyl clean up workers (14) many years after Chernobyl accident using FISH analysis. In these studies, comparable individual dose estimations were obtained with conventional dicentric analysis five-to eight years after radiation accident.

In 1987, a radiation accident occurred in Goiania Brazil. 249 individuals were exposed either internally or externally to Cs-137 gamma rays from a therapeutic source. Natarajan et al (15) compared the dose estimations of Goiania victims using two different technique. They determined the frequencies of dicentrics immediately following the accident while the translocation analysis were performed from 1992 until 1995. In these analyses, they have take into account both reciprocal and terminal translocations. They found that the frequencies of translocations observed years after the radiation exposure were two to three times lower than the initial dicentric frequencies, the differences being larger at higher doses (>1Gy).

Lloyd et al. (16) examined chromosomal translocations in a person who had accidentally incorporated tritiated water 11 years ago. The translocations yield showed good agreement with the dicentric frequency, which were performed shortly after the accident, and with a translocation frequency which were measured 11 years post exposure.

Lloyd et al (16) observed that stability for translocations over an 11 year period. However Natarajan et al (15) found lower translocation frequencies in Goiana victims 5-8 years after the accident. The accident which were examined by Lloyd et al. was protracted exposure to an incorporate radionuclide which delivered a more or less uniform whole body dose of around 0.5 Gy, however most of the victims of Goiania accident were exposed either non uniform or chronically. Their doses were changing between 0.5 Gy to 7 Gy. The differences between the periods of stability of translocations may be due to differences between exposure patterns and dose level of these two study. This interpretation support by results of Lindholm et al’s study (17) that blood samples were taken at 2-4 months intervals from people who exposed during radiation accident in Estonia in 1994. Their results indicated that the yield of translocations in
peripheral blood lymphocytes remained at a constant level after whole body exposure. On the other hand in the case of partial body irradiation, the elimination of co-existing unstable aberrations reduced the translocation yield over time.

Either animal or over exposure case studies emphasize that translocations are more stable than dicentrics. However reciprocal translocations are more persistent than non-reciprocal translocations and the period of persistence of translocations depend on exposure pattern.

1. **In-vitro Studies:**

To check validation of in-vivo results, many laboratories performed in-vitro studies. One of the important in-vitro studies are dose response curve studies. Dose response curves are necessary if a biomarker is to be used for dose assessment. To be able to use FISH analysis for dose assessment and also to compare dicentric yields with either reciprocal or total translocation yields for various doses of radiation, many laboratories were established their own in-vitro dose response curves using different radiation qualities. Finnon et al established dose response curve for X ray, gamma ray using FISH analysis. They observed that for all two radiations, measured dicentric yields in the full genome, agreed well with their previous dicentric dose response curves, which were performed using conventional Giemsa staining. They also showed that the ratio of total translocations to dicentrics is about 1.15. Finnon et al suggested that, the dose response curve for translocation yields can be derived directly from the known dose-response curve for dicentrics by applying a constant factor. Pressl et al has been generated dose response curve for X rays using FISH analysis. They indicated that because of the relatively high background level, the lowest detectable radiation dose using FISH technique is about 0.3 Gy in subjects younger than 40 years of age and about 0.5 Gy in subjects older than 40 years age. Lucas et al investigated effects of age on alpha coefficient of dose response curves. In that study peripheral blood lymphocytes from two donors, (donor 1 age 35, donor 2 age 62) were exposed to tritium beta rays and 4 donors (donors 3 and 4’s age were 24 and donors 5 and 6 were 73 and 79 years old respectively) blood samples were exposed to Co-60 gamma rays at various doses. They did not observed significantly differences on the slope of the curves.

If the FISH analysis would have been use for dose assessment it is necessary to find an answer for two important questions “Which type of translocations should have score” and “Which chromosomes should have paint”.

When considering the use of FISH in retrospective dosimetry, it is precisely known that two types of translocations, reciprocal translocations, and terminal translocations, should have use. To understand their behaviour through mitosis many studies were performed. Pale et al analysed translocation in human lymphocytes which were exposed to 2 and 4 Gy X ray after 72 h culture time to investigate the transmissibility of translocations through M1 to M2. They observed that reciprocal translocation yields for each doses stay stable however a clear
decrease were seen on terminal translocation yield. This result is in good agreement with Guerrero-Carbajal et al (23) and Hoffmann et al (24).

Guerrero-Carbajal et al (23), extended culture time from 48 to 72 and 96 for lymphocytes which were exposed to 4 Gy X rays, while Hoffman et al (24) extended culture time from 48 to 70 and 94 for lymphocytes which were exposed to 1.5 and 3 Gy X rays. Pala et al (22) and Guerrero-Carbajal et al (23) used chromosome cocktail, which is including chromosome 2,3,5, while Hoffman et al (24) used chromosome cocktail, which is including chromosome 1,2,4. The results were compared in table 1.

Table 1. The comparison of different studies on the transmission of terminal and reciprocal translocations through M1 to M2 (full genome corrected values were given)

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<thead>
<tr>
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<th>RT</th>
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<tr>
<td>1.5 Gy*</td>
<td>0.18±0.03</td>
<td>0.16±0.03</td>
<td>0.20±0.03</td>
<td>0.11±0.02</td>
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<tr>
<td>2 Gy **</td>
<td>0.22±0.03</td>
<td>0.21±0.04</td>
<td>0.22±0.03</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>3 Gy*</td>
<td>0.31±0.05</td>
<td>0.64±0.03</td>
<td>0.26±0.02</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>4 Gy **</td>
<td>0.84±0.15</td>
<td>1.00±0.15</td>
<td>0.84±0.15</td>
<td>0.65±0.12</td>
</tr>
<tr>
<td>4 Gy***</td>
<td>0.84±0.16</td>
<td>1.18±0.19</td>
<td>0.95±0.22</td>
<td>0.84±0.07</td>
</tr>
</tbody>
</table>

* Hoffman et al (24)  
** Pala et al (22)  
***Guerrero-carbajal et al. (23)

As seen on table 1 reciprocal translocation yield is not changed through M1 to M2 while terminal translocation yield is decreased. These three studies were also investigated dicentric yields. They observed a sharp decrease on dicentric yields through M1 to M2 for each doses.

In-vitro studies were also performed in order to understand is there an imbalance between sensitivity of different chromosomes, Finnon et al (18, 19) labelled chromosome 2,3,5 for their dose response curves studies and they found that there is no strong evidence for an imbalance between sensitivity of these chromosomes. Boei et al (25) were performed a study to investigate differential involvement of chromosomes 1 and 4 in the formation of chromosomal aberrations human lymphocytes after 2 Gy X-irradiation. They observed relative overrepresentation of chromosome in the formation of exchange aberrations. Translocations yield \([T(\text{Ab})+T(\text{Ba})]\) of Chromosome 1 was found as 0.095±0.005 while translocation yield of chromosome 4 was found as 0.099±0.005 in M1 cells. However, because of the differences of DNA content of these two chromosomes, the difference between yields is increased after full genome...
correction. Full genome corrected yields are 0.63±0.03 and 0.82±0.05 for chromosome 1 and 4 respectively. On the other hand the total translocation yield \([T(\text{Ab})+T(\text{Ba})]\) for chromosome 1 plus chromosome 4 for 2 Gy X-ray were found as 0.74±0.03. Although chromosome 4 is %20 over represent, translocation yield \([T(\text{Ab})+T(\text{Ba})]\) of the combination of chromosome 4 and 1 were in good agreement with 0.66±0.06 value of Pala et al’s study.

In vitro studies results were also indicate that reciprocal translocations are more persistent than either dicentrics or terminal translocations.

**CONCLUSION**

Chromosome aberration analysis has been using dose assessment for many years. It is the most convenient technique that can be use for this purpose. The lowest detectable radiation dose using dicentric analysis is about 0.1 Sv of low LET radiation and 0.01 Sv for fission spectrum neutrons while using FISH technique is about 0.3 Gy in subjects younger than 40 years of age and about 0.5 Gy in subjects older than 40 years of age. However, the decline on dicentric frequency in a time of period is limited their usage for retrospective dosimetrical purpose.

The new analysis technique, FISH, which is based on analysis of translocations in chromosome labelled metaphases, is suggested for dose assessments for extended and old exposures. Two types of translocation, reciprocal translocations, and terminal translocations are using for dose estimation. Studies showed that reciprocal translocations yield is very close to that dicentric yield immediately after exposure and this translocation’s yield persist after several cell cycles. A decrease on terminal translocation yield was observed with time after exposure. This result is indicating that reciprocal translocations are better indicator for retrospective studies than terminal translocations.

On the other hand, in the case of partial body irradiation, the elimination of co-existing unstable aberrations reduced the translocation yield over time. This situation is limited using FISH method for dose estimation of partial body exposure cases.

Results from studies of the literature show that FISH analysis could be use for dose assessment for old exposures, however, further in-vivo studies of various types of old exposures such as whole-body or partial, chronically or acute exposures at high and low doses are required to clarify whether it is confident for biological dosimetrical purpose.
REFERENCES


Clonal Aberrations for Retrospective Dose Estimation.” Int. J. Radiat Biol. 1995, 68,