ORIGINAL ARTICLE

Sporadic chromosome translocation frequencies in lymphocyte cultures – a retrospective study in a cohort of patients from Bosnia and Herzegovina

Sanin Haverić[1], Anja Haverić[1], Maida Hadžić[1], Tamara Ćetković[1], Lejla Čaluk Klačar[1], Rifat Hadžiselimović[1,2]

[1]Institute for Genetic Engineering and Biotechnology, University of Sarajevo, [2]Academy of Sciences and Arts of Bosnia and Herzegovina; Sarajevo, Bosnia and Herzegovina

ABSTRACT

Aim Chromosome translocations are considered as one of the most severe forms of genome defects. Because of the clinical significance of chromosome translocations and scarce data on the incidence of sporadic translocations in population of Bosnia and Herzegovina, we aimed to report sporadic translocation frequencies in samples karyotyped in our laboratory.

Methods The study group consisted of 108 samples. Whole blood was cultivated in complete medium for 72 hours with the thymidine application at 48th hour to synchronize the cell culture. Metaphases were arrested by colcemid 60 minutes before harvesting. Following hypotonic treatment, cells were fixed and cell suspension was dropped on coded slides. Dried slides were subjected to conventional GTG (G-banding with trypsin-Giemsa) banding and analyzed under 1000x magnification in the accordance with ISCN (International System for Human Cytogenetic Nomenclature) and E.C.A. Cytogenetic Guidelines and Quality Assurance.

Results The incidence of all detected sporadic translocations was 27.81 x 10^{-4} per metaphase. The incidence of sporadic translocations involving chromosomes 7 and 14, being considered as the most frequent sporadic translocations of the human karyotype in phytohaemagglutinin (PHA) stimulated lymphocytes, was 15.89 x 10^{-4} per metaphase. The most frequent breakpoints were 7p21, 14q11 and 14q21. Other detected sporadic translocation breakpoints were: 1q25, 3p22, 7p13, 7q11.22, 7q33, 14q23 and 19q13.4.

Conclusion Higher incidence of sporadic translocations compared to the similar studies was registered. Since potential explanations for this issue are smaller sample size and higher exposure of examined population to genotoxic agents, further monitoring of sporadic translocation incidences is recommended.

Key words: chromosome aberrations, chromosome breakpoints, genome instability

Corresponding author:
Sanin Haverić
Institute for Genetic Engineering and Biotechnology, University of Sarajevo
Zmaja od Bosne 8, 71000 Sarajevo,
Bosnia and Herzegovina
Tel: +387 33 220 926;
Fax: +387 33 442 891;
E-mail: sanin.haveric@ingeb.unsa.ba
ORCID ID: https://orcid.org/0000-0002-2999-3021

Original submission:
03 August 2021;
Revised submission:
25 October 2021;
Accepted:
04 November 2021
doi: 10.17392/1423-21

INTRODUCTION

Chromosome translocations derive from a multiple unrepaired DNA double strand breaks (DSBs) followed by a series of mistakes in cellular mechanisms for reparation or elimination of such events (1). DSBs may arise spontaneously through replication errors, exogenous stress or from scheduled breaks induced during development of the adaptive immune system (2). Unrepaired DSBs may lead to the translocation of the chromosome portion to a different chromosome - forming derivative chromosomes. Depending on the DSBs locations, translocations may cause genes fusion, or may disrupt a gene or its regulatory sequence (1). Therefore, chromosome translocations are considered as one of the most severe forms of genome defects. Chromosome translocations may appear as a consequence of exposure to various chemical (3) or physical agents (4) and have significant clinical importance since they are associated with numerous human cancers and non-cancerous diseases (5). Although chromosome translocations have long been considered mostly relevant to haematological cancers, their importance in solid tumours has been recognized as well (5).

Sporadic chromosomal structural aberrations are not randomly distributed in human karyotype. Chromosomal rearrangements resulted after translocations are considered as the most important molecular cause of various cancers (6). Numerous studies confirmed chromosome 7 and chromosome 14 translocations to be the most frequent sporadic translocations in phytohemagglutinin (PHA) stimulated human lymphocytes. In the study conducted on 11915 consecutive patients and 37 normal controls, Dewald et al. revealed that overall frequency of 7;14 translocations is 4.94×10^{-4} per metaphase (7). *In vivo* 7;14 translocations in leukocytes are considered as premalignant change ultimately leading to lymphoid neoplasia (8). Compared with the other human chromosomes, chromosome 7 is more prone to intrachromosomal duplication that, along with the evolutionary asymmetry between the long and short arms, demonstrates the dynamic nature of this chromosome with the possible adverse effects (9). Human chromosome 7 has persistently gained prominent attention also because of the frequent aberrations associated with the various forms of cancers, microdeletions within 7q11.23 are associated with Williams' syndrome and the location of the cystic fibrosis gene (9).

Because of the clinical significance of chromosome translocations and scarce data on the prevalence of sporadic translocations in population of Bosnia and Herzegovina (B&H), we aimed to report sporadic translocation frequency in samples karyotyped at the Institute for Genetic Engineering and Biotechnology, University of Sarajevo and to compare it with the previously reported studies.

PATIENTS AND METHODS

Patients and study design

The study group consisted of 108 samples of patients subjected to karyotyping analysis, mostly due to the infertility issues. Karyotyping was performed at the Laboratory for Cytogenetics and Genotoxicology of the Institute for Genetic Engineering and Biotechnology of the University of Sarajevo in the period of 2010-2020. Blood samples were collected in lithium heparinized tubes (Greiner Bio-One GmbH, Kremsmünster, Austria) by venepuncture. Whole blood cultures were initiated within 2 hours.

This retrospective study was approved by the Ethics committee of the Institute for Genetic Engineering and Biotechnology of the University of Sarajevo (No. 565/20) and conducted according to Helsinki Declaration.

Methods

Whole heparinized blood (400 µL) was added in 5 mL of PB–MAX™ Karyotyping Medium (GIBCO-Invitrogen, Carlsbad, CA, USA) and cultivated in 15mL tubes at 37°C. Thymidine (Sigma-Aldrich, St. Louis, MO, USA) was added 48 hours post culture initiation to synchronize cell culture. Cultivation lasted for additional 24 hours and metaphases were arrested by colcemid (GIBCO-Invitrogen, Carlsbad, CA, USA) with the final concentration of 0.18 µg/mL for 60 minutes before harvesting. After hypotonic treatment with 0.075 M potassium chloride (25 minutes at 37 °C), cells were fixed by immersion three times into a fresh ice-cold absolute ethanol/glacial acetic acid fixative (3:1, v/v), and final cell suspension was dropped on coded slides. Dried slides were stained by conventional GTG banding (G-banding with trypsin-Giemsa) with the resolution of 400-550 bands per haploid chromosome set.

Slides were analyzed under a BX51 microscope (Olympus, Tokyo, Japan) with 1000x magnification. Images of aberrant metaphases were documented with a DP50 digital camera (Olympus, Tokyo, Japan). Analysis was performed in accordance with An International System for Human Cytogenetic Nomenclature – ISCN (10) and E.C.A. Cytogenetic Guidelines and Quality Assurance (11).

Statistical analysis

Proportion comparison analysis was conducted to compare incidence of detected sporadic translocations in our study with those previously published. The values were considered significant at $p<0.05$.

RESULTS

Karyotyping of blood samples of 108 individuals included in this study revealed sporadic translocations in six (5.6%) individuals, equally distributed among males and females. Number of analyzed cells ranged from 15, in samples with normal karyotype, to up to 60 if aberrations were detected. The most prominent were translocations involving chromosomes 7 and 14 that were detected in 5 out of 2517 analyzed metaphases. In one blood sample, the same translocation t(7;14)(p21;q21) was found in two cells, out of 60 cells analyzed. In addition, chromosome 7 was involved in one observed translocation t(7;19)(q33;q13.4). Translocation t(1;3)(q25;p22) was also singly detected (Table 1).

Table 1. Detected translocations in peripheral blood lymphocytes (PBLs) of 108 patients included in the study

Six patients with detected tranlocations	Aberrant karyotype	Aberrant/analyzed cells per sample
1	46,XX,t(7;14)(p13;q11)	1/25
2	46,XY,t(1;3)(q25;p22)	1/60
3	46,XY,t(7;14)(p21;q21)	2/60
4	46,XX,t(7;14)(q11.22;q11)	1/40
5	46,XY,t(7;19)(q33;q13.4)	1/50
6	46,XX,t(7;14)(p21;q23)	1/50

The most frequent breakpoint was 7p21, found in three cells of two individual samples. Subsequently, single cell with 14q11 breakpoint was registered in two samples while breakpoint 14q21 was found in two cells of one individual. Other sporadic translocation breakpoints (1q25, 3p22, 7p13, 7q11.22, 7q33, 14q23 and 19q13.4) were detected only once (Figure 1).

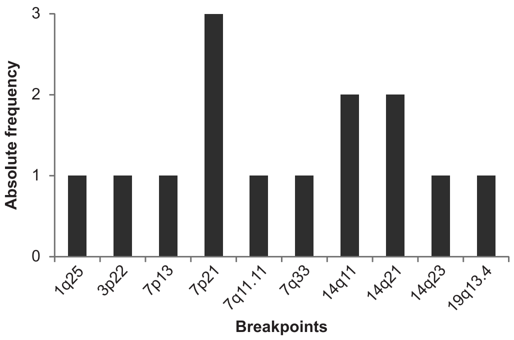

Figure 1. Ratio of detected breakpoints in 2517 observed cells of 108 patients

For all included samples (108) the total of 2517 cells were analysed. Combined incidence of all detected sporadic translocations was 27.81×10^{-4} per metaphase (7 of 2517), while incidence of sporadic translocations involving chromosomes 7 and 14 was 15.89×10^{-4} per metaphase (five of 2517). Along with the detected sporadic translocations in individuals with normal karyotype, two individuals were detected with karyotypes 46,XX,21ps+ and 46,XX,1qh+ that are considered as a normal chromosomal variants, and one individual with 47,XX,+21.

DISCUSSION

The most frequent breakpoint of chromosome 7 and all detected breakpoints in our study was 7p21. The most frequent breakpoints of chromosome 14 were 14q21 and 14q11 which is in accordance with the earlier studies (7,12). In the 48h lymphocyte cultures Hecht et al. (12) found one rearrangement per 1218 lymphocytes, and the breakpoints were exclusively 7p13, 7q35, 14q11, and 14q32. Compared to other similar studies that confirmed chromosomes 7 and 14 breakpoints as the most common in PHA stimulated lymphocyte cultures with the incidence of 5×10^{-4} (7) and 8.21×10^{-4} (12), we found higher incidence. Proportion comparison revealed a significant increase ($p=0.02$) only when compared to the incidence reported in Dewald et al. (7). Elevated incidence of sporadic translocations could be a result of smaller sample size or exposure of Bosnian population to different environmental genotoxins that is reported earlier (13-15). Environmental exposure to different xenobiotics can increase the frequencies of chromosome aberrations (16) and accordingly translocations in lymphocytes. Since they are reliable biomarker of exposure and effect, translocations are often chosen as an endpoint in human exposure studies (17). Zeljezic et al. (18) revealed an elevated frequency of chromosome translocations in a group of plant workers exposed to pesticides. Moreover, Baccarelli et al. (19) revealed a higher frequency of t(14;18) which is associated with non-Hodgkin's lymphoma (NHL) among healthy individuals from Italy exposed to dioxin that is known as NHL-associated carcinogen. The frequency of 14;18 translocation showed a significant association between occupational exposure to pesticides among farmers from Jordan (3). Genome-wide studies of translocation capture sequencing in lymphocytes revealed that translocation breakpoints are frequently positioned near transcription start sites of active genes (20,21). Chromosomal translocation, involving chromosome 14 along with chromosome 8 is characteristic for patients with Burkitt's lymphoma (22). Genes involved in this translocation are associated with the *MYC* proto-oncogene located at the chromosome 8 which is under the control of the powerful immunoglobin heavy chain gene (*IGH*) promoter on chromosome 14 (23,6). An association of acquired chromosome 14 translocations and inversions with several types of tumours (primarily hematological malignancies) has been confirmed by the karyotyping and loss of heterozygosity (LOH) studies (24). Medical radiation procedures can also induce chromosome aberrations including translocations. Cytogenetic follow-up study of Livingston et al. that was conducted on a male patient who received radioiodine treatments suggests that stable chromosome aberrations such as translocations and inversions can be useful not only for retrospective studies but also for long-term monitoring of chromosomal instability (4). Therefore, the limitation of our study is the lack of information about possible environmental and medical exposures of patients to the potential chromosomal aberrations inducers.

In conclusion, presented study contributes to the knowledge of cytogenetic status of B&H population. Results showed a higher incidence of sporadic translocations compared to similar studies. Since potential explanations to this issue are smaller sample size and higher exposure to genotoxic agents, further monitoring of sporadic translocation incidences is recommended.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflicts of interest: Nothing to declare.

REFERENCES

1. Meaburn KJ, Misteli T & Soutoglou E. Spatial genome organization in the formation of chromosomal translocations. Semin Cancer Biol 2007; 17:80-90.
2. Dudley DD, Chaudhuri J, Bassing CH, Alt FW. Mechanism and control of V(D) J recombination versus class switch recombination: similarities and differences. Adv Immunol 2005; 86:43-112.
3. Qaqish BM, Al-Dalahmah O, Al-Motassem Y, Battah A, Ismail SS. Occupational exposure to pesticides and occurrence of the chromosomal translocation t(14;18) among farmers in Jordan. Toxicol Rep 2016; 3:225-9.
4. Livingston GK, Ryan TL, Smith TL, Escalona MB, Foster AE, Balajee AS. Detection of simple, complex, and clonal chromosome translocations induced by internal radioiodine exposure: a cytogenetic follow-up case study after 25 Years. Cytogenet Genome Res 2019; 159:169-81.
5. Mitelman F, Johansson B & Mertens F. The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer 2007; 7:233-45.
6. O'Connor C. Human chromosome translocations and cancer. Nature Education 2008; 1:56.
7. Dewald GW, Noonan KJ, Spurbeck JL, Johnson DD. T-Lymphocytes with 7;14 Translocations: frequency of occurrence, breakpoints, and clinical and biological significance. Am J Hum Genet 1986; 38:520-32.
8. Reddy KS, Thomas IM. Significance of acquired nonrandom 7/14 translocations. Am J Med Genet 1985; 22:305-10.
9. Hillier LW, Fulton RS, Fulton LA, Graves TA, Pepin KH, Wagner-McPherson C, Layman D, Maas J, Jaeger S, Walker R, Wylie K, Sekhon M, Becker MC, O'Laughlin MD, Schaller ME, Fewell GA, Delehaunty KD, Miner TL, Nash WE, Cordes M, Du H, Sun H, Edwards J, Bradshaw-Cordum H, Ali J, Andrews S, Isak A, Vanbrunt A, Nguyen C, Du F, Lamar B, Courtney L, Kalicki J, Ozersky P, Bielicki L, Scott K, Holmes A, Harkins R, Harris A, Strong CM, Hou S, Tomlinson C, Dauphin-Kohlberg S, Kozlowicz-Reilly A, Leonard S, Rohlfing T, Rock SM, Tin-Wollam AM, Abbott A, Minx P, Maupin R, Strowmatt C, Latreille P, Miller N, Johnson D, Murray J, Woessner JP, Wendl MC, Yang SP, Schultz BR, Wallis JW, Spieth J, Bieri TA, Nelson JO, Berkowicz N, Wohldmann

PE, Cook LL, Hickenbotham MT, Eldred J, Williams D, Bedell JA, Mardis ER, Clifton SW, Chissoe SL, Marra MA, Raymond C, Haugen E, Gillett W, Zhou Y, James R, Phelps K, Iadanoto S, Bubb K, Simms E, Levy R, Clendenning J, Kaul R, Kent WJ, Furey TS, Baertsch RA, Brent MR, Keibler E, Flicek P, Bork P, Suyama M, Bailey JA, Portnoy ME, Torrents D, Chinwalla AT, Gish WR, Eddy SR, McPherson JD, Olson MV, Eichler EE, Green ED, Waterston RH, Wilson RK. The DNA sequence of human chromosome 7. Nature 2003; 424:157-64.

10. Shaffer LG, Tommerup N. ISCN 2005; An International System for Human Cytogenetic Nomenclature. Basel: S Karger, 2005.
11. Hastings R, Howell R, Bricarelli FD, Kristoffersson U, Cavani S. General guidelines and quality assurance for cytogenetics: a common European framework for quality assessment for constitutional, acquired and molecular cytogenetic investigations. Neuilly-sur-Seine, France: E.C.A. - European Cytogeneticists Association, 2012; E.C.A. Newsletter; no 29.
12. Hecht F, Hecht BK, Kirsch IR. Fragile sites limited to lymphocytes: molecular recombination and malignancy. Cancer Genet 1987; 26:95-104.
13. Krunić A, Haverić S, Ibrulj S. Micronuclei frequencies in peripheral blood lymphocytes of individuals exposed to depleted uranium. Arh Hig Rada Toksikol 2005; 56:227-32.
14. Ibrulj S, Haverić S, Haverić A, Durmić-Pasić A, Marjanović D. Effect of war and postwar genotoxins on micronuclei frequency in Sarajevo study group. Bosn J Basic Med Sci 2006; 6:54-7.
15. Besic L, Muhovic I, Asic A, Kurtovic-Kozaric A. Meta-analysis of depleted uranium levels in the Balkan region. J Environ Radioact 2017; 172:207-17.
16. Ibrulj S, Haverić S, Haverić A. Chromosome aberrations as bioindicators of environmental genotoxicity. Bosn J Basic Med Sci 2007; 7:311-6.
17. Tucker JD. Chromosome translocations and assessing human exposure to adverse environmental agents. Environ Mol Mutagen 2010; 51:815-24.
18. Zeljezic D, Vrdoljak AL, Lucas JN, Lasan R, Fucic A, Kopjar N, Katic J, Mladinic M, Radic B. Effect of occupational exposure to multiple pesticides on translocation yield and chromosomal aberrations in lymphocytes of plant workers. Environ Sci Technol 2009; 43:6370-7.
19. Baccarelli A, Hirt C, Pesatori AC, Consonni D, Patterson DG, Bertazzi PA, Dölken G, Landi MT. t(14;18) translocations in lymphocytes of healthy dioxin-exposed individuals from Seveso, Italy. Carcinogenesis 2006; 27:2001-7.
20. Chiarle R, Zhang Y, Frock RL, Lewis SM, Molinie B, Ho YJ, Myers DR, Choi VW, Compagno M, Malkin DJ, Neuberg D, Monti S, Giallourakis CC, Gostissa M, Alt FW. Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. Cell 2011; 147:107-19.
21. Klein IA, Resch W, Jankovic M, Oliveira T, Yamane A, Nakahashi H, Di Virgilio M, Bothmer A, Nussenzweig A, Robbiani DF, Casellas R, Nussenzweig MC. Translocation-capture sequencing reveals the extent and nature of chromosomal rearrangements in B lymphocytes. Cell 2011; 147:95-106.
22. Bozkurt S, Okay M, Saglam F, Haznedaroglu IC. Retrospective evaluation of chromosome 1 anomalies in hematologic malignancies: a single center study. Genetics and Applications 2019; 3:17-24.
23. Rowley JD. Chromosome translocations: dangerous liaisons revisited. Nat Rev Cancer 2001; 1:245-50.
24. Kamnasaran D, Cox DW. Current status of human chromosome 14. J Med Genet 2002; 39:81-90.