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Original article

Telomere length and chromosomal fragility increase in car painters exposed to organic solvents

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ABSTRACT

Objective: To comparing the chromosome fragility ratio and relative telomere length, of a group of car painters occupationally exposure to organic solvents with a group of non-exposed individuals in the city of Bogotá (Colombia).
Methods: This was a retrospective study of two cohorts (33 men each), matched by age (±2 years) at 1:1 ratio. The differences in the fragility ratio and relative telomere length distributions were determined for both groups. Case group was composed for adult males working in artisanal shops located in Bogotá (Colombia), who were occupationally exposed to organic solvents through automotive painting for a minimum of two years. Blood samples were analyzed. The chromosomal fragile sites were detected using cytogenetics techniques and DNA was extracted to determine the length of telomeres using qPCR (Cawthon's method). Chromosomal fragility ratio and relative telomeric length were compared between car painters exposed to organic solvents and no-exposed individuals.
Results: Statistically significant differences in Chromosomal fragility ratio were found between exposed (0.645 ± 0.440, med = 0.520) and non-exposed (0.414 ± 0.217, med = 0.400) (p = 0.037, Wilcoxon one-tailed test). Also, in relative telomere length, in exposed (2.728 ± 5.581, med = 1.668) and non-exposed (1.835 ± 4.727, med = 0.732) individuals (p = 0.002, Wilcoxon one-tailed test). Low degree correlation for the two parameters, using Spearman's Rho, was found (0.21, p = 0.419).
Conclusions: Exposure to organic solvents increase both the telomere length and the rate of chromosomal sites fragile in the exposed group compared to the unexposed group. This study is a pioneer in the evaluation of the genotoxicity of exposure to organic solvents obtained the chromosomal fragility ratio and measuring the relative length of telomeres. We suggest applying this methodology to workers in other types of industries, for example carpentry shops, paint factories and stores where organic solvents are mixed and there is occupational exposure due to the inadequate use of protection measures or space conditioning.

Abbreviations: APH, Polymerase inhibitor aphidicolin; ATM, Ataxia-telangiectasia mutated; ATR, ATM- and Rad3-Related; BTX, Benzene, toluene and xylene; CF, Chromosome fragility; CFR, Chromosomal fragility ratio; CFS, Chromosomal fragile sites; EDTA, Ethylenediaminetetraacetic acid; FISH, Fluorescence in situ hybridization; HBG, Human beta-globin; hESC, human embryonic stem cell; HPBMC, Peripheral blood mononuclear cell; IARC, International Agency for Research on Cancer; IR, Risk index; NHL, non-Hodgkins lymphomas; PAH, Polycyclic aromatic hydrocarbons; PAHs, polycyclic aromatic hydrocarbons; POT1, Protection of Telomeres 1; RPMI, Roswell Park Memorial Institute; RTL, Relative length of telomeres; TADs, Topologically associated domains; TL, Length of telomeres; TLV, Threshold Limit Value; TRF1, Telomeric Repeat Factor 1; TRF2, Telomeric Repeat Factor 2; UVC, Ultraviolet light between 200–280 nm.

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1. Introduction

Volatile organic solvents such as benzene, toluene and xylene (BTX) are substances which can negatively affect human health. Workers in different industries are exposed at those compounds and suffer deterioration of health. BTX are widely used in the manufacture of automotive paints, are including in solvent mixtures such as thinner (Martinez and Martinez, 2016). The BTX are fat soluble, highly flammable and volatile at room temperature (Jeanne Mager Stellman et al., 1998). International Agency for Research on Cancer (IARC) bulletins categorize benzene as a carcinogen; toluene as a possible carcinogen and xylene has not been proven to be carcinogenic (WHO I Cancer Today, 2012).

Exposure to organic solvents can cause irritation of the respiratory tract or skin, drowsiness, headache, nausea and vomiting. In the long term, it can produce damage to the nervous system and kidneys, heart rhythm abnormalities, shortness of breath, anemia, and even leukemia. In general, most human life-sustaining systems may be affected. The health complications caused by the BTX airborne exposure also can be associated with kidney and bladder cancer, some leukemias, anemias, hematocrit alterations and decrease white blood cell number (Hadkhale et al., 2017).

Genotoxic agents cause DNA damage, which are detected using molecular and cytogenetics techniques. In the case of occupational exposure to organic solvents in paints, the following techniques have been used: micronuclei, comet assay, measurement cell-free DNA; also cytogenetic techniques for detecting chromosomal aberrations as sister chromatid exchange, dicentric chromosomes and chromatid exchanges. Others cytogenetic techniques identify chromosomal fragile sites (CFS), which are a cytogenetic phenomenon capable of generates genomic instability by inducing structural changes of DNA that alter the processes of replication, transcription and even may cause mutations that lead to the development of diseases (Hellman et al., 2002). CFS are inherited chromosomal regions that are observed as gaps, constrictions or breaks on metaphase chromosomes when cell under culture are treated with DNA replication inhibitors. The human genome database documents more than 120 CFS. There is evidence of genetic damage related to increased concentration of solvent metabolites in urine after exposure to these compounds. BTX are genotoxic, proven to cause DNA alterations, which may generate mutations or even interfere with the systems responsible for its repair (Quintero et al., 2009).

Other source of genomic instability and accumulation of DNA damages is the chromosome ends length (telomeres). The telomere degradation dynamic is intrinsic, however external factors such as exposure to physical or chemical agents may alter this equilibrium, causing extension or erosion of the telomeres. Therefore, telomere length (TL) is an important indicator of the proliferative capacity of human tissues cells (Tümpel and Rudolph, 2012). TL is dynamic and results from a balance between shortening and lengthening of telomeres. Also is a complex heritable trait as well as genetic variations that influence telomere maintenance, therefore is a potential biomarker of the impact of environmental conditions on telomeric DNA. The length of human telomeres decreases with each cell division until a critical point where subtelomeric genes are exposed or altered, this situation causes chromosomal instability, leading to cell death or aberrant recovery of proliferative activity. In the latter case, a high error rate during replication may be induce, which increases genetic instability and favors produce mutations which could result in oncogenes activation or inhibition of tumor suppressors genes, those events are known to promote carcinogenesis. The telomere shortening can be influenced by a host of factors related to physical and mental health, lifestyle factors, exposure to pollutants, gender, ethnicity as well as cellular factors such as oxidative damage and replicative stress caused by genetic, epigenetic, and environmental factors. Telomere shortening has been found to be associated with obesity (Buxton et al., 2011), diabetes (Salpea et al., 2010), some cancers (Ennour-Idrissi et al., 2017; Renner et al., 2018), and cardiovascular disease (Fitzpatrick et al., 2007). Also, with exposure to pollutants such as those produced by tobacco. Differences by gender have been reported, where females having longer telomeres compared to males (Gardner et al., 2014).

On the other hand, the environmental and occupational exposure to pollutants may increase the replication rate, so is to be expected reduce the telomere length in a shorter period than usual. Those compounds, cause cumulative oxidative stress and chronic inflammation which in turn are pathways involved in carcinogenesis induction (Hou et al., 2012). The telomere shortening was associated to exposition to benzene/toluene, black carbon particles, polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, pesticides and chemical mix in car mechanical workshops, also with esophageal and skin cancer and non-Hodgkins lymphomas (NHL) (Torres et al., 2008). Otherwise an telomere elongation was reported with short exposition (at least three days) to metal particles and arsenic, which may induce biological changes, including oxidative stress and chronic inflammation (Hou et al., 2012). Other studies have demonstrated an association between long telomeres and increased risk of cancer such as melanoma, basal cell carcinoma, glioma, lung, tumors of the urogenital system, lymphoma (Srinivas et al., 2020). This can be explained because long telomeres are related to greater proliferative potential and replicative crisis, which in turn induced carcinogenesis. Possible sources of conflicting results are length and dose of exposure, the study design, and the possible mechanisms involved in telomere regulation, such as activation of DNA repair pathways, cell death, and cell senescence (Fig. 1).

The detection of chromosome fragilities as evidence of chromosomal abnormalities and telomere length have not been used for evaluated the genotoxicity of occupational exposure to the organic solvents presents car paints. To circumvent this point, the current study included a group of non-exposed workers and obtained the CFR, which considers all FSs observed in the blood samples in relation to the total number of metaphases, making the groups comparable in this aspect.

Given that traditional car painters are occupationally exposed to organic solvents, and this could cause genetic alterations, this study evaluated the presence of fragile sites and the length of chromosomal telomeres in a group of exposed workers and one not exposed. Chromosomal fragile sites were detected using cytogenetics techniques (Weise, 2010) and the length of telomeres was measured using qPCR based method that compares the telomeric signal with a single copy gene (T/S ratio) (Cawthon, 2002). Then, the values of chromosomal fragility ratio (CFR) and relative telomeric length (RTL) were analyzed to determine if these values are different between those groups and they are correlated with some habits such as tobacco and alcohol consumption.

2. Materials and methods

2.1. Study population

This was a blind, retrospective study of two cohorts, matched by age (±2 years), carried out in 2013. For the exposed cohort, the inclusion criteria were adult males working in artisanal shops located in Bogotá (Colombia), in the Barrios Unidos locality, who were occupationally exposed to organic solvents through automotive painting for a minimum of two years. For the non-exposed cohort, the inclusion criteria were adult males from the same locality without occupational exposure to organic solvents due to automotive painting. The exclusion clinical criteria for both cohorts

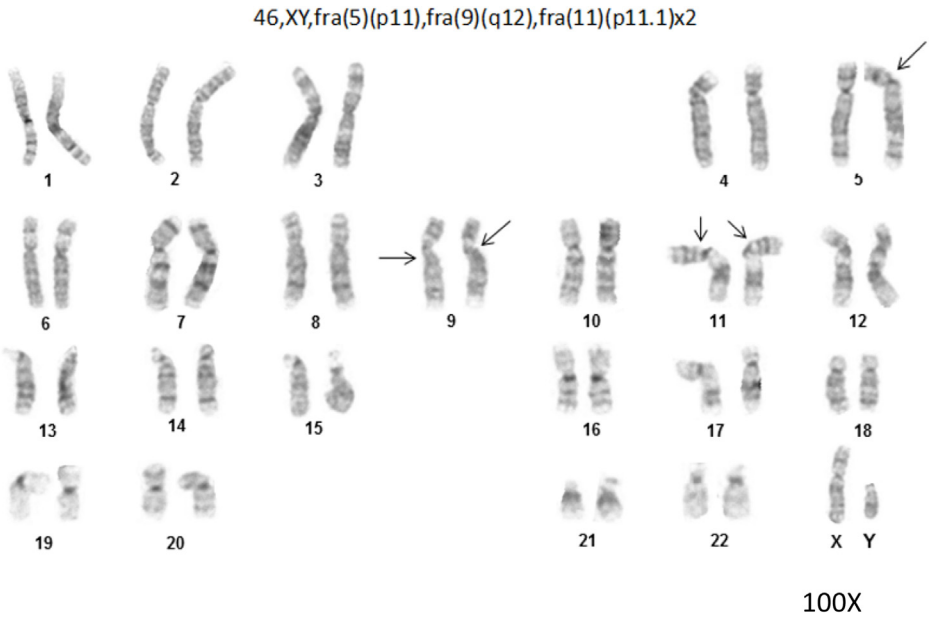

Fig. 1. Representative image of a G-banding metaphase showing fragility of chromosomes 5, 9, and 11 from a car painter worker exposed to solvents.

were a history of hepatitis, any type of cancer or medical treatment which would include radiation therapy or chemotherapy, or any protracted medical treatment. The exclusion criteria for calculating CFR was to have at least 100 metaphases of good dispersion and morphology at the discretion of the group's cytogeneticists.

2.2. Dependent variables

The chromosome fragility ratio (CFR), defined as the number of observed fragilities in an individual divided by the number of observed metaphases in the same individual (CFR = # of Fragilities/# of Metaphases).

Relative telomere length, obtained by Livak's method, or the double delta Ct method, which calculated the target sequence/single gene copy ratio relative to the reference DNA employed (Samsonraj et al., 2013). The independent variable was occupational exposure to organic solvents through automotive painting. Probable confounding variables were also identified (age, consumption of alcohol and cigarette).

2.3. Data collection

Data was collected using a questionnaire which was applied directly to the study subjects from both cohorts and included the variables of length of exposure to organic solvents (years), use of personal protective equipment for work, and habits such as smoking and alcohol consumption.

Participation was voluntary for individuals in both groups, and an informed consent was signed. This study adhered to the ethical standards of the Declaration of Helsinki for human subject research (de Mazzanti Di Ruggiero, 2011). The ethics committee of the Universidad del Rosario reviewed and approved this study. The data supplied by the participants were handled with total confidentiality.

Blood samples were obtained from every-one, drawn by aseptic venipuncture. One heparinized 5 ml tube for the chromosome fragility test and one EDTA 5 ml tube for DNA extraction and telomere length measurement.

2.4. Chromosomal fragile sites

The chromosomal fragile sites were obtained using cytogenetic standard protocols lymphocyte cultures following the indications of Weise et al 2010 (Weise, 2010). Four slides were observed under a light microscope and 100 metaphases per each participant, after taking pictures with the regular stain, the slides were discolored with Carnoy's solution for 2 min and the G-banding process was applied and those showing chromosome with fragile sites (FS) were photographed.

2.5. Measurement of relative telomere length (RTL)

Lymphocyte DNA was extracted from 350 µl of the blood sample, following the instructions of the DNA 2000 kit manufacturer (Corpogen), designed exclusively for extracting human DNA from blood samples. After qPCR was performed, and the data were processed using Cawthon's method as standardized by Samsonraj et al. (2013), then the RTL was obtained using Livak's method cited by Samsonraj et al. (2013). Each sample was processed in triplicates and a standard curve was obtained with human embryonic stem cell (hESC) DNA in serial dilutions. In addition, the DNA of two people, one 25-year-old and one 55 year-old, was used as the internal control for telomere length, as well as MCF-7 cell line DNA.

2.6. Statistical analysis

Descriptive analysis of the quantitative variables was provided through central tendency (mean and median) and dispersion (range and standard deviation) measures. The CFR and the RTL were evaluated with the Shapiro-Wilk test. The smoking and alcohol consumption habits were analyzed using the asymptotic or exact McNemar test (expected values < 5) for related samples.

The QPCR data had a standard deviation < 0.8 and coefficient of variation < 10 %. The series values of each assay were transferred to a spreadsheet (MS-Office-Excel 2013 v.15.0) to obtain the trend graph of the data, each graph's equation and its corresponding coefficient of determination, R2. The equation presented for each

graph allows the expected Ct value for a 10 ng DNA concentration to be calculated.

The study design consisted of two related samples matched for age, between the cohorts, with a 1:1 ratio. The differences in CFR and RTL distributions were evaluated using the nonparametric one-sided Wilcoxon rank test for related samples. Spearman's (two-sided) correlation coefficient was used to determine the degree of correlation between CFR and length of exposure to organic solvents, and between RTL and length of exposure to organic solvents. The difference between CFR and RTL in the exposed and non-exposed groups with respect to alcohol and cigarette consumption was compared using the Mann-Whitney *U* test (one-sided). A 95 % confidence level was established, and the SPSS 22 program (V8.500021 Universidad del Rosario) was used for all statistical analyses.

3. Results

3.1. Cohort characteristics

The study cohorts were controlled for age by matching and were comparable. No significant differences between the cohorts regarding smoking (p = 0.687, McNemar exact test), alcohol consumption (p = 0.219, McNemar exact test) and the average length of alcohol use (p = 1.000, paired *t*-test). (Table 1).

3.2. Chromosomal fragility

In 16 of the 33 individuals from the exposed cohort, chromosome fragilities were not able to be analyzed for not having enough cell growth to complete the 100 metaphases with good dispersion and morphology that were required to be included in the analysis. The CFR was measured in 17 pairs (exposed with non-exposed), finding high heterogeneity among the values with the coefficient of variation (49.3 % and 64.2 %, respectively). The CFRs were significantly greater in those exposed (0.645 ± 0.440, med = 0.520) than in the non-exposed (0.414 ± 0.217, med = 0.400) (p = 0.037, Wilcoxon one-tailed test).

Table 1
Demographic and epidemiological characteristics of exposed and non-exposed individuals.

Variable	Exposed (n:33)	Non-exposed (n:33)
Age (mean ± SD*; years)	42.71 ± 14.83	42.88 ± 15.07
Age (med; years)	42	41
Interquartile range	52	53
Age (min - max; years)	20 – 73	21 – 73
Length of exposure to organic solvents (mean ± SD; months)	234.33 ± 141.38	
Smoking habit (%)		
Non-smoker	84.8 %	78.8 %
Smoker	15.2 %	21.2 %
Length of time smoke-free (mean ± SD*; months)	218 ± 142.47	128.67 ± 97.24
Length of time smoke-free (med; months)	150	116
Interquartile range	312	276
Alcohol consumption (%)	97(%)	84.8(%)
Length of alcohol consumption (mean ± SD; months)	226.33 + 149.73	279.50 ± 172.21
Length of alcohol use (med; months)	186	246
Interquartile range	588	696

* SD: Standard Deviation.

3.3. Relative telomere length (RTL)

Telomere length measurements showed an extremely high variation between those exposed (CV = 204.6 %) and not exposed (CV = 257.6 %). There were differences in telomere length, which were significantly greater in exposed (2.728 ± 5.581, med = 1.668) than in non-exposed (1.835 ± 4.727, med = 0.732) individuals (p = 0.002, Wilcoxon one-tailed test).

The correlation between CFR and length of exposure to solvents, using Spearman's Rho, showed a low degree of correlation (0.21, p = 0.419). In the same way a weak correlation between RTL and length of exposure to solvents, was found (−0.388; p = 0.026), considering the ratio range. The negative sign in the correlation indicates an inverse correlation between length of exposure and RTL. There was a moderate correlation between the CFR and the RTL (−0.064; p = 0.808). The Mann-Whitney *U* test was used to compare consumption habits (alcohol and cigarettes) for each group with CFR and RTL, the difference was not statistically significant; the values are shown in Table 2.

4. Discussion

This study focused in detection of DNA damage on car painters, with laboral exposure to organic solvents, those who works in the informal market and have a limited or inadequate use of personal protective equipment frequently (Palma et al., 2015a; Varona-uribe and Groot-restrepo, 2007). To our knowledge, this is the first study using the CFR and RTL to determine genotoxicity to laboral exposure to BTX, also an unconventional statistical methods to evaluated the statistical significance of comparation the exposed versus unexposed groups workers and their correlated with some habits such as tobacco and alcohol consumption.

A greater frequency of fragilities was found in the genetic material of exposed individuals to solvents, compared to those not exposed (p = 0.037). Interestingly, the most frequent site fragile was located on 9q12.9, similar to other studies related to breast cancer in women (Rondón-Lagos et al., 2006), and in bladder cancer cells, where the loss of heterozygosity of the 9q12 to 9q31 region has been described, which includes the tumor suppressor PTCH gene (Williams et al., 2002). Additionally, the SPATA31A5 and SPATA31A7 members of FAM75A (former designation) gene family are in the same band. Those genes encode proteins having a nuclear localization signal and high expression in cells exposed to UVC light and human testis where they are a role in spermatogenesis. The primate protein SPATA31 has a domain that suggest a possible function in UV response and DNA repair (Bekpen et al., 2017). Also, a recurrent fragile site in 9q12 has been characterized in epithelial cells and registered on band 9q12 in the "Atlas of Genetics and Cytogenetics in Oncology and Hematology". Several chromosomal abnormalities in 9q12 band have been described, such as del (9) (q12-32), related to acute myeloid leukemia, der17 t(9;17)(q12-p11) associated to myeloma multiple, du(9) (q12q12) found in carcinoma ductal tumor among others. In the exposed, 274 different types of fragilities were detected, while in

Table 2
Relationship between CFR, RTL and alcohol and tobacco consumption habits in exposed and non-exposed individuals to car paint solvents.

Consumption habits, by group	CFR (p value)	RTL value (p value)
Exposed group		
Alcohol	−0.816 (0.294)	−0.105 (0.485)
Smoking	−0.596 (0.309)	−1.105 (0.145)
Non-exposed group		
Alcohol	−0.630 (0.296)	−1.004 (0.338)
Smoking	−1.193 (0.147)	0.220 (0.846)

the unexposed there were 69. The two groups shared 39. The ten most frequent were 7.8 % fra(9)(q12.9), 4.1 % fra(16)(p11.2), 3.8 % fra(11)(p11.2), 3.5 % fra(1)(q12), 2.6 % fra(10)(q11.2), 2.6 % fra(12)(p11.2), 2.3 % fra(7)(p13), 2.3 % fra(7)(q11.2), 2 % fra(2)(p11.2) and 2 % fra(2)(q11.2). Among these, those that contain genes with roles in proliferation, DNA repair or activation of cell death that could have a role in cancer are fra(11)(p11.2), fraa(10)(q11.2), fra(7)(p13), fra(2)(q11.2).

Under normal conditions, the presence of repetitive and regulatory elements in fragile sites does not produce genomic instability, but they were related to early replication regions that are delayed under replication stress. The replication time (RT) program is tissue specific and associated with chromatin architecture and transcriptional activity, so (RT) per se is not a reason for the expression of the fragile site in lymphocytes, although in fibroblasts it was shown that it may be cause a delayed replication time in G2 or even in mitosis. Therefore, tissue-specific epigenetic and transcriptional programs appear to be the final contributors towards chromosomal breakage development. It was found that the presence of fragile sites depends on both the cell type and the stress inducer, which suggests that the presence of fragility is affected by cellular events such as DNA replication, transcription, and the organization of the genome such as topologically associated domains (TADs) (Sarni et al., 2020). The Sarni et al determined a signature of chromosomal fragility after replication stress induced by the DNA polymerase inhibitor aphidicolin (APH), which revealed the presence of TAD border regions that include genes with high transcriptional activity and large size that induce a delay in the replication timing (RT) (Sarni et al., 2020). Also common FS are part of the signature of cancer genomic instability, because they are found in precancerous cells as an effect of replicative stress (Irony-Tur Sinai and Kerem, 2019). Exposure to solvents could cause replicative stress that delays DNA replication to G2 or mitosis and induces the expression of fragile sites.

The relationship between CFR and length of exposure to organic solvents was low (p = 0.419). In contrast, Moro et al. (2012) suggest an increased genetic damage associated to length of exposure, when toluene concentrations is low. This difference could be related to the concentration of solvents and/or their metabolites in the organism of the exposed individuals.

The current study RTL was greater in exposed individuals, regardless of length of exposure, which was not a determining factor (p = 0.026, this can delayed the activation of senescence or apoptosis, giving the cell more time to accumulate mutations that increase the risk of carcinogenesis (Lan et al., 2009). As has been pointed out, RTL is inversely related to greater biological age, which is consistent with the findings of the current study where PC (25 years old) had a greater RTL than LC (50 years old). Likewise, the results showed that the correlation between length of exposure and RTL is inverse; as length of exposure to solvents increases, RTL tends to decrease, consistent with what was reported by Müezzinler et al. (2013). However, exposure to environmental benzene levels more than 31 ppm, was related to increased telomere length (1.37+/−0.23), adjusting for age and sex (p = 0.03), compared to group control in three factories that used products containing benzene in Shanghai (Bassig et al., 2014). Although we do not have measurements of the environmental levels of solvents, in a previous study (Palma et al., 2015b), they measured benzene, xylene and toluene particles using the NIOSH 1500 method, in 11 workshops where the car painters worked, in the same sector of the city of Bogotá (Colombia), where the samples were taken for this study. The level of benzene was lower than 0.5 ppm in all of them, in 4 of 11 the toluene was higher than 20 ppm and xylene exceeded (100 ppm). In the same study the respective risk index (IR) for each solvent, was calculated by dividing the value of the chemical substance by its Threshold Limit Value (TLV) with four hours of exposure. The IR of toluene and xylene in 4 workshops were above the biological exposure limit, with a risk index greater than 1 and 2 of them have the IR of benzene close to 1. Despite this, there is evidence of subtle, subclinical, and prepathological early renal and hepatic dysfunction in individuals exposed to BTX at sub-TLV limit levels (Neghab et al., 2015).

Benzene was ratified by the IARC in the year 2012, as a carcinogen (group1) to human, and has been linked to the development acute myeloid leukemia and acute non-lymphocytic leukemia (WHO I Cancer Today, 2012), skin cancer on hands and forearms (Stenehjem et al., 2017) as well as lymphomas and leukemias (Lan et al., 2013). Benzene and toluene combining have been related to bladder cancer (Hadkhale et al., 2017). The IARC considers automotive painters have a more risk for cancer due to occupational exposure to solvents than no exposed (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans et al., 2010). It has been reported that lifestyle could be a factor that mitigates or increases the risk of getting sick, in this study was no correlation between consumption of alcohol or cigarettes with CFR (p = 0.29 and p = 0.31, respectively), in opposition with the findings by Bogadi A. et al. who found a strong correlation between cytogenetic findings and consumption of alcohol but not cigarettes (Bogadi-Šare et al., 1997). The differences are probably due to other factors related to health status and ethnicity, not analyzed. Therefore, the studies are not comparable due to differences in the gender of the participants and the experimental techniques used. They included 49 female workers in a shoe factory, added bromodeoxyuridine (BrdU) to culture medium and observed 200 metaphase per individual under the microscope. Torres et al. led a study with male and female individuals, and found significant difference according to consumption of alcohol or cigarettes (Torres et al., 2008), they used micronucleus and comet assay techniques to measure genotoxicity. Analysis of the relationship of TL and gender are contradictory in the literature, however a systematic review and meta-analyses conducted by Gardner et al concluded that average females had longer telomeres than males and the strength of these associations varied by measurement method but not by age group (Gardner et al., 2014). It should also be noted that blood cells comprise a mixture of different individual cell types with different mitotic history, then telomere length can exhibit a large intra-individual variability.

This study determined an increase in CFR and in the RTL in the group of exposed individuals compared to not exposed. These tests can be useful to detect subtle changes that occur at the cellular level before the clinical manifestations of possible diseases related to BTX exposure, so the measurement of telomere length is a good candidate to be used as biomarker of genomic instability. In addition, it provides useful information to generate and implement management and control strategies for the elimination and reduction of environmental pollutants in auto paint service workshops.

Also, the current study suggests that exposure to automotive paint may decrease both de number of lymphocyte and their adaptive capability to be growing under the culture conditions, because 16 out of 33 samples were unable to have enough metaphases to make the analyzes. In this sense Rashnuodi et al reported a decrease of white blood cells count in humans due to exposure to xylene(Rashnuodi et al., 2021) and Parvez et al found suppression of T-cell proliferation by polycyclic aromatic hydrocarbons (PAH) and arsenic exposure dose dependent (Parvez et al., 2019). Moreover, Lauer et al described changes in human peripheral blood mononuclear cell (HPBMC) populations and T-cell subsets with an increased in the percentage of T helper cells (Lauer et al., 2019).

5. Limitations and suggestions

This study is a pioneering research on the use of CFR and RTL as indicators of genotoxicity by exposure to BTX in informal car pain-

ters, so do more works with a major participants number to be increase the power is required. However, the resolution of the G bands to identify CF is limited, so the detection of fragility is underestimated, so it is recommended to complement this type of study with techniques such as FISH. Also, could be investigate the effects on the immune system similar to Lauer et al and Parvez et al studies (Lauer et al., 2019).

We suggest applying this methodology to workers in other types of industries, for example carpentry shops, paint factories and stores where organic solvents are mixed and there is occupational exposure due to the inadequate use of protection measures or space conditioning. In addition, a cohort study is suggested in which individuals may be followed on at least two occasions during a time of exposure to have a comparative measurement. A baseline would be established at the beginning of the study, to recording changes, over time, of chromosome fragilities and length of the telomeres as well as health complications or symptoms diseases.

6. Conclusion

This study is the first to evaluate RTL related to occupational exposure to organic solvents in Colombia. The data showed that exposure to automotive paint may increase individuals' RTL as well as the frequency of chromosomal fragilities compare with no exposed individuals. In addition, no statistically significant correlation was found between CFR and RTL.

The current study did not show a correlation between length of exposure to solvents and increased chromosomal fragilities and RTL in the group of automotive painters. However, it is worth noting that it is important to explore this aspect using another type of variable which would allow this correlation to be determined, for example, level of metabolites in urine and concentration of solvents in the workplace. Telomere length in white blood cell DNA may be a biomarker of future increased risk of lung cancer in diverse populations measure circulating leukocyte telomere length.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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