RESEARCH ARTICLE

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Chromosome Abnormalities and Absolute Telomere Lengths of Leukocytes from Silk Weavers with Emphasis on Potential Genotoxicity and Mutagenicity of Silk Dyes

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Abstract

Objectives: This study is aimed to assess the possible genotoxicity and mutagenicity of silk dyes on silk weavers. **Methods:** Peripheral blood leukocytes were obtained from 24 silk weavers and 24 age- and sex-matched controls in northeastern Thailand. After mitogen stimulation in culture, chromosome abnormalities were examined using Giemsa banding and the absolute telomere length (aTL) was measured with SYBR green qRT-PCR. To confirm genotoxic and mutagenic effects of silk dyes, leukocytes from one each of healthy male and female volunteers were cultured with various concentrations of 3 dark red silk dyes under the presence of mitogen. Chromosome abnormalities and the telomere length were determined as above. **Results:** The proportion of normal metaphase in the silk weaving workers was significantly lower than that in controls. The frequency of chromosome aberrations was higher in the silk weavers than in control group. Polyploidy was detected only in the silk weavers. The aTL was significantly shorter in the silk weavers than in control group ($p < 0.05$). When leukocytes from normal volunteers were stimulated with mitogen under the presence of various concentrations of 3 silk dyes, suppressed the mitotic index (MI) and normal metaphase, whereas the proportion of prophase and the incomplete chromosome forming increased significantly. All dyes induced polyploidy. Dye #CA5 induced structural changes in male leukocytes, whereas #30 induced the changes in female leukocytes. The #CA5 increased aTL of normal leukocytes in a dose-dependent manner. **Conclusions:** All dyes, especially #CA5, have high genotoxicity and mutagenicity to induce chromosome aberrations and telomeric instability. Taken all those results together, regular health checking of silk weavers who have been exposed to those dyes is critically necessary to prevent various chemical-induced carcinogenesis.

Keywords: Silk dye- genotoxicity- chromosome- telomere-qRT-PCR

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Introduction

In Thailand, traditional fabric weaving is a popular source of income earning among rural women, especially in the Northeastern area because it allows them to work and attend to their families at the same time. However, those silk weavers often suffer from health problems not only due to physical constraints but also to be exposed to various chemicals including silk dyes (Nilvarangkul et al., 2006). Recently in addition to natural dyes, some synthetic acid-dyes (azo dyes) have been widely used for silk dyeing. Various synthetic textile dyes are known to have genotoxicity and mutagenicity (Tsuboy et al., 2007; Ferraz et al., 2011; Soriano et al., 2014; Leme et al., 2015). Previous study showed untreated silk dyeing waste have genotoxic effects on murine bone marrow cells (Chaurasia et al., 2005). Because azo dyes contain heavy metals such as chromium and cobalt, they can cause chromosome aberrations such as aneugeny at physiological concentrations (Figgitt et al., 2010). Recently, using micronucleus cytome assay, significantly higher incidence of nuclear anomalies including karyolysis, karyorrhexis, condensed chromatin, and pyknosis have been found in exfoliated buccal cells of cotton workers in small weaving household factories in Pakistan (Khan et al., 2015). Binazzi et al., (2015) have made a systematic review and meta-analyses on the relationship between occupational exposure and sinonasal cancer, they mentioned that occupational exposure to formaldehyde, nickel and chromium

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compounds in textile industry or construction would increase the risk of sinonasal carcinoma.

Synthetic silk dyes are mostly acid or azo dyes and some of them contain chromium metal. In Thailand, the popular dyes used in silk industries are dark red dye commercial products including #30, #CA5 and #46. Silk weavers often use the conventional open-air method for dyeing processes without any awareness of the need for self-protection. Therefore, the aims of this study were to assess the possible hazardous effects of silk dyes on silk weaving workers in northeast Thailand. For this purpose, we examined two biomarkers, chromosome abnormalities and the absolute telomere length (aTL), of peripheral blood leukocytes of silk dye-exposed subjects and unexposed (control) subjects in the same environment. In this study, in addition to chromosome aberration, aTL was used as the biomarker because aTL has been commonly used as a genotoxic and mutagenic marker for diagnostic purpose (Aubert et al., 2011). Also, to confirm the genotoxicity and mutagenicity of silk dyes routinely used in silk weaving factories in northeast Thailand, the effects of three representative red silk dyes on the chromosomes and aTL of normal leukocytes from healthy volunteers were examined in vitro.

Materials and Methods

Study subjects and blood samples

Among silk-weaving women residing in a famous Thai Silk-Weaving village, Kum Kee Tao, Thumbon Chonnabot, Chonnabot District, Khon Kaen Province, Thailand, 24 subjects who are supposed to have been exposed to silk dyes for at least for 5 years were selected. The equal number (24) of age- (44 to 76 years old) and sex-matched women who have other professions but living in the same environment were selected as controls. Interviews were conducted using questionnaires for potential influence evaluation, and demographic characteristics were collected from both groups. All participants signed the informed consent before interview and blood collection. From all participants, 5 mL each of blood samples were collected by venipuncture and transferred to sterile sodium heparin vacutainer tube in an icebox and transported to our laboratory.

As a separate experiment, to evaluate genotoxicity and mutagenicity of silk dyes in vitro, sterile 20 mL of heparinized blood samples from one each of healthy male and female volunteers, both 54 years of age, were obtained by venipuncture and were transported to our laboratory as mentioned as above.

Blood culture for karyotype analysis and absolute telomere length determination

Heparinized whole blood (0.5 mL) of each participant was added to 5 mL of RPMI 1640 medium supplemented with L-glutamine (Gibco™ by Life Technology), containing 10% fetal bovine serum (Gibco™ Invitrogen) and 100 U/mL of penicillin, 100 µg/mL streptomycin. To stimulate G0 stage of lymphocytes, 4% phytohemaglutinin (PHA M-form Gibco™ by Life Technology) was added to the culture. The cultures were incubated at 37^{O}C for 72 h in a humidified incubator with 5% CO_2 atmosphere.

To determine genotoxicity and mutagenicity of silk dyes in vitro, various concentrations (10-500 µg/mL) of three most popular dark red textile dyes used in Thai silk weaving factories, #30, #CA5, and #46, were added to the blood culture of healthy male and female volunteers. The cultures were incubated at 37^{O}C in a humidified incubator with 5% CO_2 atmosphere. All three dark red silk dyes examined were azo dyes and were sent to the Central Laboratory (Thailand) Co., Ltd., for heavy metal analysis.

Chromosome preparation and analysis

Giemsa (G)-banding was performed according to the standard method (Yunis and Sanchez, 1973). A hundred mitotic cells were examined under a microscope at x1,000 magnification. Metaphase images were analyzed using the MetaSystem software. The proliferation status of cultured cells was defined as the mitotic index (MI = the sum of all cells in prophase, metaphase, anaphase and telophase per 1,000 cells) (Medri et al., 2003). The aberrant chromosome patterns were defined as the percentage of cells, according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016 (McGowan-Jordan et al., 2016).

Determination of absolute telomere length (aTL)

Absolute telomere length (aTL) was determined using SYBR Green quantitative real time polymerase chain reaction (SYBR green qRT-PCR). Briefly, genomic DNA of cultured cells were extracted and purified using PureLink® Genomic DNA Kit (InvitrogenTM) according to the manufacturer's protocol. The extracted DNAs were kept at -80oC until use. Quantitative real-time amplification of telomere sequence was performed according to the previously described methods (O'Callaghan and Fenech, 2011) with slight modification to optimize the condition.

The oligomers used for PCR reaction to establish the standard curve were the forward primer ggTTTgTTTgggTTTgggTTTgggTTTgggTTTgggTT-3′), and the reverse primer (5′-ggCTTgCCTTA CCCTTACCCTTACCCTTACCCTTACCCT-3′) (O'Callaghan et al., 2008).

Standard curve establishment

The 20 µL amplification reaction mixture contained dH2O 4.8 µL, 1x SYBR Green, 2.0 µM forward primer, reverse primer (20 µM) 0.1 µL and DNA template 5 µL with 6 dilutions. The PCR reaction was performed using the LightCycler® 480 real time PCR system (Roche Diagnostics GmbH, Mannheim, Germany) with the following program. Preincubation at 95^{O}C for 10 min followed by 32 cycles of amplification with denaturing at 95^{o}C for 15 sec, annealing at 60^{o}C for 1 min and extension at 72^{o}C for 20 sec followed by melting curve for PCR product identification 1 cycle with 95^{o}C for 10 sec, 65^{o}C for 1 min, 98^{o}C continuously. The final step was cooling with 40^{O}C for 10 sec. The standard curve Figure 1 was established using serial dilution of known quantities of synthesized 84 mer oligonucleotide with a MW of 26667.3 consisted of TTAGGG repeats 14 times (TIB MOLBIOL,

Syntheselabor GmbH, Germany).

Statistical analysis

Statistical analysis was performed using an Excel Statistic add-in program. While ANOVA-Two-Factor Without Replication was used for the data analysis of chromosome abnormalities of normal blood cells cultured with silk dyes, Wilcoxon Signed Rank test was used for chromosome abnormalities of the silk weaving workers and control non-silk weaving workers. Absolute telomere length (aTL) results from normal blood cells cultured with various concentrations of #30, #CA5 and #46 were analyzed using regression analysis. Wilcoxon Signed-Rank test at 95% confidence was used to compare the aTL of silk weaving and control groups.

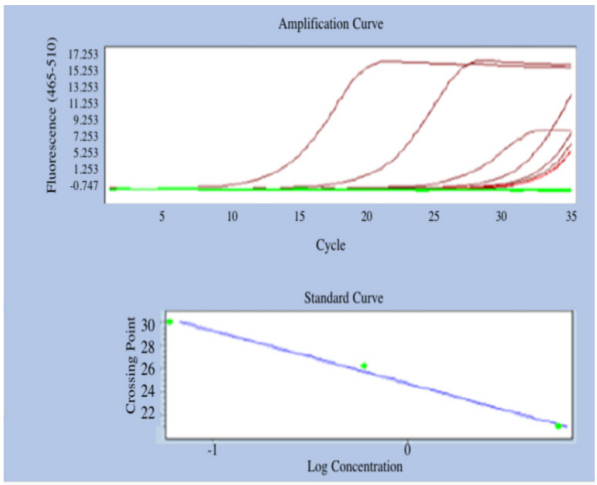
Ethical clearance

This research was reviewed and approved by the International Ethics Board, Khon Kaen University, Thailand (Reference No. HE561012).

Results

Chromosome analyses for silk weaving workers and control subjects

Demographic characteristics of 24 silk weaving workers and 24 control subjects were shown in Table 1. The aberrant chromosome patterns found in this study were staying in prophase stage, incomplete chromosome forming, polyploidy Figure 2a-2c. The results of chromosome analyses of mitogen-stimulated cultured leukocytes of the silk weaving workers and control subjects were summarized in Table 2. The MI was not significantly different statistically (p-value > 0.05, Wilcoxon Signed-Rank test) between two groups. However, the proportion of normal metaphase in the silk weaving workers was significantly lower than that of control subjects (p-value < 0.05). The percentages of prophase (cell division inhibition) and incomplete chromosome forming were significantly higher in the silk weaving works than in the control subjects (p-value < 0.05). Polyploidy of chromosomes were found only in the silk weaving workers group.



Crossing Point (CP)	Standard
7.74	6.00E2
12.62	6.00E1
30.00	6.00E0

Figure 1. Standard Curve of SYBR Green qRT-PCR, Generated by LightCycler® 480 Real Time PCR System (Roche Diagnostics GmbH, Mannheim, Germany), for Telomere Length Determination Using Known Quantities of Synthesized 84 Mer Oligonucleotide with a MW of 26667.3 Consisted of TTAGGG Repeats 14 Times (TIB MOLBIOL, Syntheselabor GmbH, Germany).

Absolute Telomere length (aTL) of the silk weaving workers and control subjects

Absolute telomere lengths (aTL) of the silk weaving workers and control subjects were determined using SYBR Green qRT-PCR. The results are shown in Table 3. Obviously, the silk weaving workers had significantly shorter (p-value < 0.001 by Wilcoxon Signed-Rank test) aTL than did the control subjects Table 3. However, neither the aTL of the silk weaving workers nor control subject groups correlated to the ages of the study subjects (p-value = 0.975; Regression analysis).

Effects of silk dyes on normal leukocyte chromosomes in vitro

Since we have found significantly higher chromosome abnormalities and shortened aTL of the mitogen-stimulated cultured leukocytes of the silk weaving workers compared with control subjects, we have investigated the mutagenicity of silk dyes on mitogen-stimulated normal lymphocytes from healthy volunteers. The results of chromosome patterns were summarized in Figure 3. All three red silk dyes, #30, #CA5 and #46, had suppressed MI and normal metaphase of normal human leukocytes (p-value < 0.05; ANOVA: Two-Factor Without Replication).

The proportions of metaphase of normal leukocytes were significantly suppressed by three dyes of every concentrations. Conversely, the proportion of prophase (cell division inhibition) tended to increase by the addition

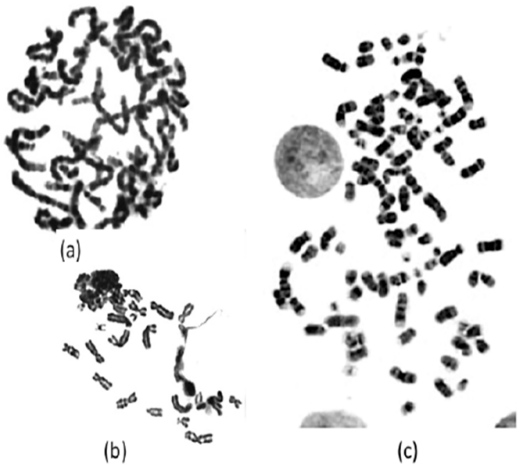


Figure 2. Chromosome Change Patterns Observed in Normal Leukocyte Cultured with Silk Dye and in Silk Dye Exposed Subjects, (a) Prophase (cell division inhibition), (b) Incomplete Chromosome Forming, and (c) Polyploidy.

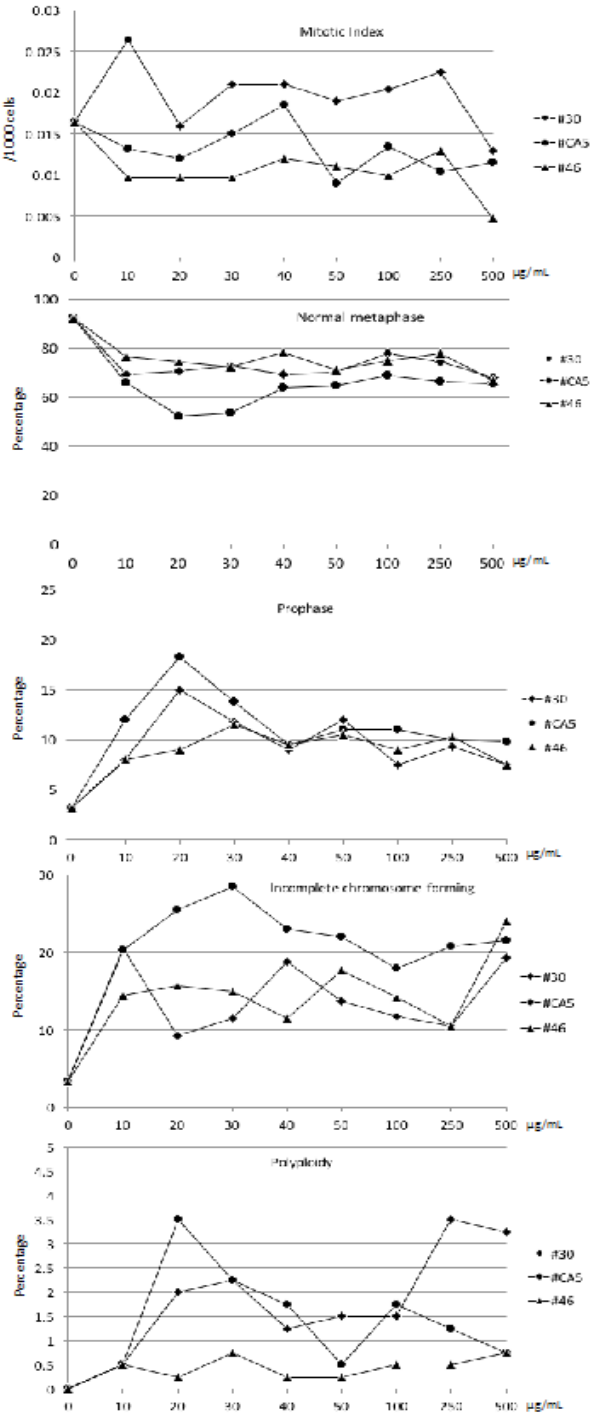


Figure 3. Mitotic Index and Chromosome Ppatterns Observed in Normal Human Mitogen-stimulated Leukocytes Under the Presence of Silk Dyes #30, #CA5 and #46.

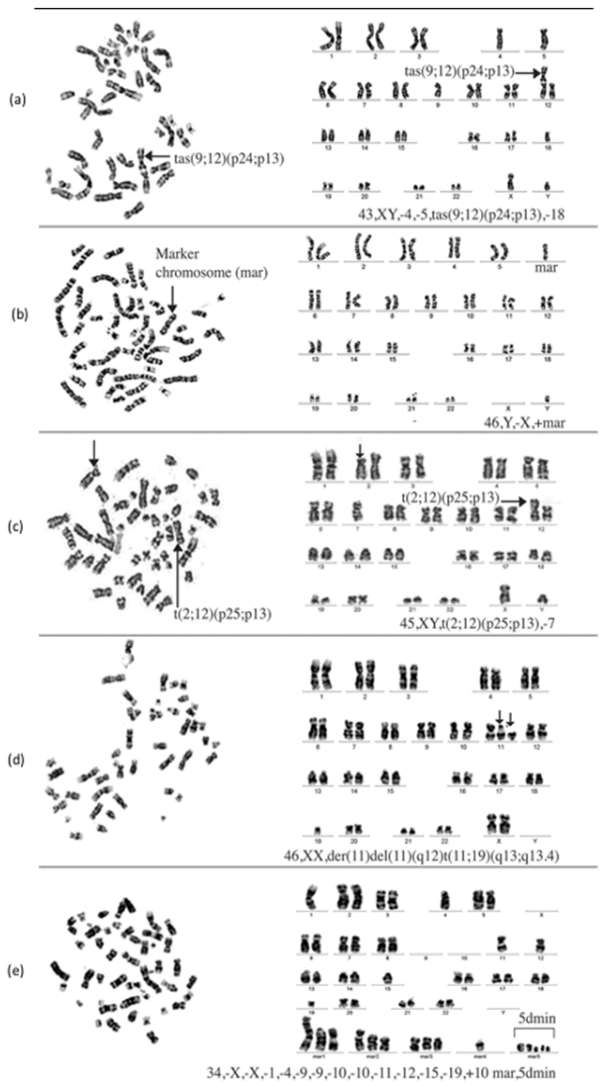


Figure 4. The Chromosome Structural Abnormalities Found in Male and Female Leukocytes Induced by Silk Dye #CA5 and #30, (a) Incomplete Chromosome with Telomeric Association BetweenChromosome 9 and 12 [43,XY,-4,-5,tas(9;12)(p24;p13),-18], (b) Chromosome Loss and Rearrangement with 46,Y,-X,+mar, (c) Chromosome Loss and Rearrangement with 45,XY,t(2;12)(p25;p13),-7, (d) the Structural Chromosome Aberrations 46,XX,der(11)del(11)(q12) t(11;19)(q13;q13.4) and (e) 34,-X,-X,-1,-4,-9,-9,-10,-10,-11,-12,-15,-19,+10 mar,5dmin.

of silk dyes to the culture and were significantly different among the concentration of dye added (p-value < 0.05; ANOVA: Two-Factor Without Replication). Chromosome aberrations such as incomplete chromosome forming and polyploidy were induced by silk dyes. A few structural changes of chromosomes were observed in various combination of the doses of dyes and male and female leukocytes. The silk dye #CA5 at 50 µg/mL induced 43,XY,-4,-5,tas(9;12) (p24;p13),-18 in male leukocytes (Figure 4a) and the same dye at 250 µg/mL induced 46,Y,-X,+mar and 45,XY,t(2;12) (p25;p13),-7 Figure 4b and 4c. For female leukocytes, the silk dye #30 at 250 µg/mL induced 46,XX,der(11)t(11;19) (q13;q13.4)del(11)(q12q13) and 34,-X,-X,-1,-4,-9,-9, -10,-10,-11,-12,-15,-19,+10 mar,5dmin Figure 4d and 4e.

Effects of silk dyes on normal leukocyte aTL in vitro

When the effects of three silk dyes, #30, #CA5 and #46, on the aTL of mitogen-stimulated normal leukocytes from healthy donors were examined, only #CA5 caused dose-dependent increased of aTL of normal human leukocytes (R2 = 0.64185) as shown in Figure 5.

Major heavy metal contents in the silk dyes

Central Laboratory (Thailand) Co., Ltd., reported that three silk dyes, #30, #CA5, and #46, used in this study

Table 1. Demographic Characteristics of 24 Silk Dye Exposed and 24 Control Subjects

Characteristics	Exposed subjects		Control subjects	
	No.	%	No.	%
Sex				
Female	24	100	24	100
Age				
< 50 years	7	29/17	7	29/17
≥ 50 years	17	70/83	17	70/83
Status				
Single	3	12/5	3	12/5
Married	14	58/33	8	33/33
Widow	7	29/17	13	54/17
Education				
Primary school	21	87/5	11	45/83
Lower secondary school	2	8/33	3	12/5
High school	1	4/17	5	20/83
Post-secondary college	0	0	1	4/17
Bachelor's degree	0	0	4	16/67
Income				
< 3,000 baht (~86 USD)	18	75	10	41/67
≥ 3,000 baht ((~86 USD))	6	25	14	58/33
Occupation				
Silk spinning, dyeing, weaving, mudmeea	4	16/67	0	0
Silk spinning, dyeing, weaving	1	4/17	0	0
Silk dyeing, weaving, mudmee	10	41/67	0	0
Silk dyeing, weaving	7	29/17	0	0
Silk dyeing, mudmee	2	8/33	0	0
Mudmee(only)	0	0	2	8/33
Weaving (only)	0	0	2	8/33
Beauty salon	0	0	2	8/33
Housewife	0	0	4	16/67
Grilled banana vendor	0	0	1	4/17
Grocery owner	0	0	3	12/5
Food vendor	0	0	2	8/33
Government officer	0	0	1	4/17
Retired government officer	0	0	2	8/33
Dress maker	0	0	2	8/33
Office employee	0	0	1	4/17
Laundry	0	0	1	4/17
Health volunteer in communities	0	0	1	4/17
Concurrent occupations				
None	13	54/17	24	100
Rice farmer	8	33/33	0	0.00
Gardener	1	4/17	0	0.00
Merchant	0	0	0	0.00
Food vendor	1	4/17	0	0.00
Health volunteer in communities	1	4/17	0	0.00
Silk weaving working age				
< 5 years	0	0	0	0
≥5 years	24	100	0	0

Table 1. Continued

Characteristics	Exposed subjects		Control subjects	
	No.	%	No.	%
Silk dye exposed duration				
< 5 years	0	0	0	0
≥ 5 years	24	100	0	0
Self-protection				
Yes	19	79.17	0	0
No	5	20.83	0	0
Dye stirring material				
Wood (or bamboo) stick	24	100	0	0
Allergic history from silk dyes exposed				
No	15	62.5	0	0
Dizziness	1	4.17	0	0
Feel dry	2	8.33	0	0
Mouth numbness	1	4.17	0	0
Dyspnea	1	4.17	0	0
Dyspnea and itchiness	2	8.33	0	0
Dyspnea and sneezing	1	4.17	0	0
Sore eyes	1	4.17	0	0

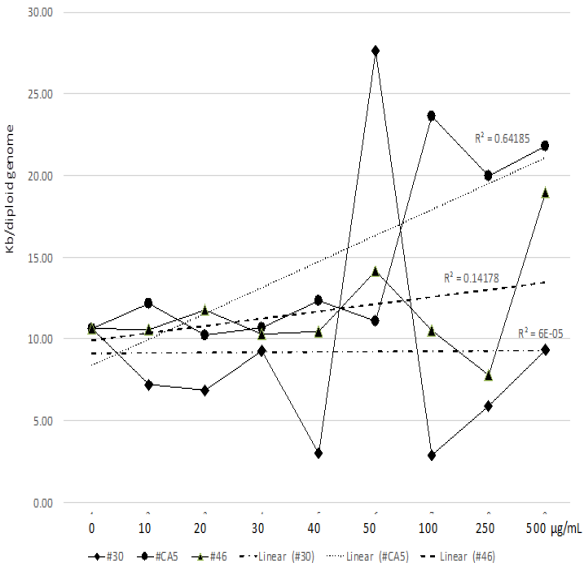


Figure 5. Linear Regression of Absolute Telomere Length (aTL) of Human Blood Cultured Cells Added with Various Doses of Silk Dyes #30, #CA5 and #46. Linear regression analysis revealed that the aTL of mitogen-stimulated human leukocytes cultured with #CA5 correlated with concentration of the dye added (R2 = 0.64185).

contained chromium (Cr) 4.00, 3318.00 and 50.67 mg/kg respectively, using in-house method based on EPA 3052 (Microwave assisted acid digestion of siliceous and organically based matrices).

Discussion

Widely used synthetic silk dyes, especially azo dyes containing heavy metals, are assumed to cause direct

Table 2. Mitotic Index and Percentage of Chromosome Patterns (Median and range) of 24 Silk Weavers (Exposed Subjects) and 24 Control Subjects

Chromosome patterns	Exposed subjects		Control subjects		p-value [a]
	Median	Range	Median	Range	
Mitotic Index [b]	0/01	0 - 0.02	0/01	0.00-0.02	> 0.05
Normal metaphase (%)	84/25	74.00 - 92.50	100	94.5 - 100	< 0.05
Prophase (%)	11/25	3.50 - 20.50	0	0 - 4	< 0.05
Incomplete chromosome forming (%)	0	0 - 4.00	0	0 - 1.50	< 0.05
(n/total)	11/24		2/24		

[a], Wilcoxon Signed Rank test; [b], he sum of all cells in prophase, metaphase, anaphase and telophase per 1,000 cells

Table 3. Average Telomere Length Crossing Point (TC) and Absolute Telomere Length (aTL) of 24 Silk Weavers (Exposed Subjects) and 24 Control Subjects

No.	Age	Average TCa		Average aTL (kb/diploid genome)[a]	
		Exposed	Control	Exposed	Control
1	65	20.02	18.4	4.59	38.1
2	64	18.44	19.01	8.98	26.53
3	69	19.52	19.64	5.66	20.3
4	63	16.93	19.57	17.13	21.07
5	50	18.73	18.65	7.96	31.07
6	63	19.74	17.57	5.16	49.17
7	76	20.88	21.96	3.18	7.53
8	50	18.8	17.78	7.72	45.13
9	70	19.04	18.89	6.95	27.9
10	70	20.84	18.58	3.23	31.9
11	49	18.96	17.5	15.7	48.23
12	49	18.68	17.85	17.73	41.57
13	59	20.25	18.86	9.07	26.97
14	50	20.04	18.99	9.91	25.5
15	44	19.69	18.88	11.5	26.77
16	52	21.58	19.2	5.14	23.4
17	61	20.44	16.91	8.37	62.17
18	46	20.31	18.97	8.84	25.8
19	66	18.87	19.89	16.37	17.43
20	56	20.41	17.81	8.51	42.43
21	60	17.68	18.11	36.63	30.43
22	68	15.62	16.38	87.1	63.67
23	60	17.1	17.73	46.87	35.9
24	58	17.2	18	44.87	31.93
Median	60	19.28	18.615	8.91	30.75
p-value[b]		< 0.05		< 0.05	

[a], An average from triplicated aTL measurements; [b], Wilcoxon Signed Rank test.

health problems and also render genotoxic and mutagenic effects on humans. According to the demographic data shown in Table 1, the silk weaving workers who are assumed to be exposed silk dyes for over 5 years had more frequent health problems such as dizziness, dry throat, mount numbness, dyspnea, skin itchiness, sneezing and sore eyes compared to the control subjects. The highest health problems were dyspnea, skin itchiness and dry throat. Similar to our results, Upadhyay et al., (2016) reported that about 40% of cloth dyeing workers in Jaipure, India, were suffered from occupational contact dermatitis. Also, Salmani Nodoushan et al., (2014) showed significantly higher incidence of acute and chronic respiratory problems among textile-dyeing workers in the cross-sectional study in Iran.

In the present study, the most important findings are the significantly higher detection rates of genotoxic damages of mitogen-stimulated lymphocytes from silk weaving workers compared to those of control subjects. In this study, the frequency of chromosome changes observed at the prophase stage inferred by cell division inhibition or incomplete chromosome forming at metaphase were significantly higher in the silk weaving workers than in controls. Such aberrant expression of chromosome changes at metaphase were reported to be seen in malignant patients (Guleria et al., 2005; Harsimran et al., 2009). When normal leukocytes obtained from healthy male and female donors were cultured with mitogen stimulation in vitro, addition of silk dyes in culture caused significant aberration of chromosomes mentioned as above for silk weaving workers. In addition, silk dyes induced significant increase of chromosome polyploidy in the cells. Polyploidy and aneuploidy were common gross abnormalities of chromosomes. Related to the present study, Chaurasia et al., (2005) suggested that the silk dye waste interfered with the internal milieu of cells leading to gross type damage of chromosomes, while certain ions and reactive radicals formed during their metabolism induced the breakage of the chromosomes.

In the present study, not only genotoxicity but also the structural chromosome aberrations were observed in normal male leukocytes cultured with 50 and 250 µg/mL of silk dye #CA5 Figure 4a, 4b, and 4c and in normal female leukocytes with 250 µg/mL of dye #30 Figure 4d and 4e. The induction of chromosome rearrangement indicates the mutagenicity of those silk dyes. Chromosome aberrations were found in several chromosome numbers and regions of the cultured normal leukocytes. Some of them had potential for molecular pathogenesis of several neoplasms. The presence of chromosome rearrangement indicates mutagenicity occurred because the genetic material alteration that can no longer be repaired by the cells. Chromosome aberrations found in blood cultured cells in this study involved several chromosome numbers and regions which many genes located on. Some of these mutations are involved in the molecular pathogenesis of various malignancies (Keene et al., 1987; Cotterill, 2015; Ullah Shah et al., 2015; Wen et al., 2015; Burandt et al.,

2016; Dickson et al., 2016; Lu et al., 2016; Ross et al., 2016).

Double minute chromosomes (Dmins) play a critical role in tumor cell genetics as they are frequently associated with the overexpression of oncogene products; they have been observed in metaphase chromosomes from tumor cells for many years under light microscope (Hahn, 1993). Dmins are the cytogenetic hallmark of extrachromosomal genomic amplification and are likely to be closely associated with genomic instability in carcinoma cells (Xu et al., 2015).

Telomere length is a critical indicator of the cells undergoing senescence and/or apoptosis. Certain agents associated with specific occupations or lifestyles may accelerate telomere shortening by inducing damage to general DNA or more specifically at telomere portions, resulting in the shortening of the lifespan of individuals. In this study, aTL of the silk weaving workers were significantly shorter than that of the control subjects. Related to this finding, Hoxha et al., (2009) evaluated the aTL of office workers and traffic police officers and found that aTL of traffic police officers were significantly shorter than office workers at each corresponding age group. They speculated that police officers might have been exposed to benzene, one of very high potential carcinogen. Pavanello et al., (2010) reported also the shorter aTL in peripheral blood lymphocytes of polycyclic aromatic hydrocarbon exposed workers, and suggested that shortened aTL could be a central event in polycyclic aromatic hydrocarbon carcinogenesis. Shortening of telomere length is known to associate with the increased risk of various cancers (Mirabello et al., 2010; Bau et al., 2013; Qu et al., 2013; Qin et al., 2014; Sun et al., 2015a). In addition, the shorter aTL in peripheral leukocyte cultured cells (mostly lymphocytes) may has been reported to be associated with a wide range of health and disease conditions. Ziegler et al., (2017) suggested that telomere shortening due to polychlorinated biphenyls exposure might lead to limitations of cell renewal and clonal expansion of lymphocytes. These events related to immune dysfunctions have been linked to increased susceptibility to infectious diseases and increased risk of cancer. In contrast, the longer telomere length was found to be associated with persistent organic pollutants (POPs) exposure such as dioxin, furans, and polychlorinated biphenyls that increased risk of cancers too (Scinicariello and Buser, 2015; Mitro et al., 2016). Our in vitro study was also found that silk dye #CA5 induced aTL extension on leukocyte cultured cells but the mechanisms was unclear.

Finally, in this study, all three silk dyes tested contained chromium at various concentrations. Chromium is one of many heavy metals that can induce chromosome instability and DNA damage (Singh et al., 1998; Blasiak and Kowalik, 2000; O'Brien et al., 2003; Hu et al., 2011). Hexavalent chromium [Cr(VI)] is labeled as carcinogen when inhaled, and oral exposure to Cr(VI) is carcinogenic in rats and mice (Sun et al., 2015b). Thus, production methods of dyes as well as the qualities of dyes should be strictly controlled and the risk of its usage should be informed to the users by proper means.

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