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Relationships Between Cytokine (IL-6 and TGF-β_1) Gene Polymorphisms and Chromosomal Damage in Hospital Workers

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Abstract

Cytokine gene polymorphisms have been found to be associated with a pre-disposition to a variety of diseases, including inflammatory and cancer diseases. The present study evaluated the influence of six cytokine gene polymorphisms on the level of genomic damage observed in peripheral blood lymphocytes from hospital pathologists chronically exposed to low doses of different xenobiotics. Lymphocytes from 50 pathologists and 50 control subjects were recruited and analyzed in Sister Chromatid Exchange (SCE) and Chromosomal Aberrations (CA) assays. The frequencies of six cytokine gene polymorphisms and their relationships with the cytogenetic damage levels were also evaluated. The results indicated that significant differences were found between pathologists and controls in terms of SCE frequency ($p < 0.001$) and RI values ($p < 0.001$), as well as in terms of CA and cells with aberrations ($p < 0.001$). No associations were found between all analyzed cytokine gene polymorphisms and CA frequency in both pathologists and control groups. *Vice versa*, among pathologists, homozygote individuals for the IL-6 G allele showed a significantly ($p = 0.017$) lower frequency of SCE with respect to heterozygote subjects. Similarly, for TGFβ_1 codon 10 locus, homozygote for T allele and heterozygote TC subjects showed a significantly ($p = 0.021$) lower frequency of SCE with respect to homozygote CC individuals. Among controls, no significant differences were found in the frequency of SCE between genotypes at all loci. Based on these results, we speculate that high circulating levels of a pro-inflammatory cytokine like IL-6 and lower levels of the immunosuppressant cytokine TGFβ_1 could be associated directly with a longer duration and/or greater intensity of inflammatory processes, and indirectly with significantly higher levels of genomic damage.

Introduction

Cytokines are a broad category of small proteins that play a crucial role in regulating all aspects of immune responses. They are produced by a wide range of cells, including immune cells, in response to interaction with specific and non-specific antigens or soluble stimulus. They are part of the immune surveillance system, exerting their function as mediators of the immune regulation and are involved in the inflammatory response. They also influence the synthesis and the action of other cytokines, leading to activation of complex cascades of cytokine network (Lazutka, 1996).

Cytokine genes are polymorphic and some of these polymorphisms, mostly single nucleotide polymorphisms (SNP) located within coding and/or regulatory regions, have been shown to affect the overall expression and secretion of the gene products, explaining individual variations in cytokine production and inter-individual differences in immune responsiveness (Uboldi de Capei et al., 2003; Hollegaard and Bidwell, 2006; van Dyke et al., 2009).

The present study evaluated levels of genomic damage in peripheral blood lymphocytes from hospital pathologists chronically exposed, for professional reasons, to low doses of different environmental xenobiotics, such as formaldehyde and ethylene oxide. These last two xenobiotics, principally used for sterilization, are well-known human carcinogens (IARC, 1988) and are related to an increase of both chromosomal aberrations (CA) and sister chromatid exchanges (SCE) among exposed subjects (Lorenti et al., 2001; Donner et al., 2010; Santovito et al., 2011).

Occupational or environmental exposure to xenobiotics could have harmful health effects depending on the duration of exposure, the type of chemical agents and the individual susceptibility. Several studies have examined the relationship between cytokine gene polymorphisms and a variety of diseases including infectious diseases and cancers (Colakogullari et al., 2008; Helmig et al., 2009; Karaoglan et al., 2009; Taherkhani et al., 2009; van Dyke et al., 2009).

Some cytokine gene polymorphisms were also found to be associated to inflammatory diseases. Genetic variants at the interleukin (IL)-10 locus have been shown to modulate innate inflammatory and the

chronic diseases responses (Smith and Humphries, 2008; Kuningas et al., 2009). Similarly, it has been demonstrated that chronic inflammatory diseases, known to be associated with cellular transformation and malignancy, are characterised by systemically elevated levels of tumour necrosis factor (TNF)-α, a pro-inflammatory cytokine with pleiotropic effects (Greten et al., 2004; Pikarsky et al., 2004; Higashimoto et al., 2006; Westbrook et al., 2012).

It is known that the level of the genomic damage due to environmental or occupational exposure to different chemicals also depend on the different individual susceptibility. From genetic point of view, this susceptibility is due to polymorphisms in a battery of genes, principally metabolic genes such as glutathione-*S*-transferase (GST) and cytochrome P (CYP) 450 family genes. However, some cytokine gene polymorphisms were also found to be associated to an increase of the genomic damage. Human interferons (IFN), TNFα, epidermal growth factor and IL-2 have been showed to exhibit genotoxic properties in human peripheral blood lymphocyte cultures, in terms of increased SCE frequency (for review, see Latzuka, 1996; Higashimoto et al., 2006; Westbrook et al., 2012).

It is our opinion that, considering their implication in the modulation of some genomic damage associated diseases, such as inflammatory and cancer diseases, the cytokines gene polymorphisms may also have a role in the amount of the genomic damage. So, we decided to evaluate, in a sample of workers chronically exposed to xenobiotics, such as the hospital pathologists, the influence of some cytokine gene polymorphisms on the level of genomic damage expressed in terms of increased SCE and CA frequencies. These two cytogenetic biomarkers have been considered to be markers of early biological effects of the exposure to carcinogens. SCE are considered as the consequence of DNA-replication errors on a damaged template occurring during interchange processes between DNA replication products at homolo-gous chromosomal loci. In general, the SCE assay could have important applications in those cases in which a defect in DNA repair is suspected (Garcia-Sagredo, 2008). On the other hand, the CA assay allows the detection of cells carrying unstable aberrations (i.e. chromosome and chromatid breaks, fragments, rings, dicentrics) that will lead to cell death during proliferation (Garcia-Sagredo, 2008). Previous studies

evidenced that increased CA frequencies in peripheral blood lymphocytes are a powerful predictor of cancer risk and could be associated with early events of carcinogenesis (Bonassi et al., 2000, 2004).

Several published studies have focused on occupational risks for hospital workers (Kopjar et al., 2009; Santovito et al., 2011, 2014) principally linked to exposure, although at low doses, to different chemicals used in their work routines. Moreover, some healthcare workers do not follow standards established by their employers, putting themselves at risk for mutagenicity (Ritchie et al., 1999). In this scenario, the use of bio-monitoring processes among personnel with potential worksite exposure is of primary interest in biological safety.

Materials and Methods

Study populations

Demographic characteristics of exposed and control populations involved in the study are reported in Table 2. This study included 50 hospital pathologists (23 males and 27 females, mean age 41.12 [± 8.08], range 25-60) working in three hospital departments and 50 control subjects (23 males and 27 females, mean age 39.86 [± 8.06], range 24-66) belonging to Administrative staff of the same hospitals - but without any work-related exposure to hazardous agents. The mean years of employment resulted 10.36 [± 7.74] for pathologists (range 1-27) and 11.34 [± 6.34] for controls (range 1-31). Pathologists were exposed to different chemicals, mainly sterilizing gases (i.e., formaldehyde, ethylene oxide), anti-neoplastic drugs, and antibiotics. All exposed subjects declared to use complete protective equipment (according to Italian guidelines) and were routinely tested for urinary and blood drug levels. A lack of data on individual exposure doses of pathologists was because the majority of the workers enrolled in this study were likely exposed to a variety of different xenobiotics and did not use dosimeters. For this reason, environmental exposure was considered as being the time (in years) the worker was exposed to different xenobiotics. Hence, this study was interested in discovering the extent of genomic damage on peripheral lymphocytes of pathologists.

It is known that drugs and radiations could influence the levels of cytogenetic damage (Santovito et al., 2013; 2014), as well as smoking and alcohol assumption may influence the production of cytokines (Szabo 1999; Shimoyama et al., 2001). For these reasons, the sample population here exclusively considered individuals who have not smoked nor consumed alcohol and drugs, and have not been subjected to diagnostic examinations for a period of at least 2 years prior to the analysis. All the subjects were healthy volunteers, received information about the study, and were extensively interviewed by a specialized physician with a detailed questionnaire in order to provide important information for the study. The procedures followed in this work were approved by the local responsible committee on human experimentation and were per-formed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Blood sample collection

Fasting blood samples for genotoxicity testing were routinely obtained from the subjects by venipuncture (5-10 ml) at 08.30 AM and collected into heparinized tubes. All samples were coded, cooled (4°C), and processed within 2 hr of collection.

SCE

Each SCE assay was performed according to Santovito et al. (2014b). To determine the number of SCE/cell for each subject, 50 well-spread second-division metaphases containing 46 (± 1) chromosomes were scored. A total of 100 cells/donor were scored for determination of the replication index (RI), calculated as: $RI = (M_1 + 2M_2 + 3M_3)/N$, where M_1, M_2 and M_3 represent the number of cells undergoing first, second, and third mitosis and N is the total number of metaphases scored.

CA assay

Each CA assay was performed according to Santovito et al. (2011). For each subject, a total of 200 well-spread first-division complete metaphases were analyzed for the following categories of CA: chromatid

breaks (B'), chromosome breaks (B"), dicentrics (Dic), acentric fragments (AF), rings (R) and tri-/tetra-radials (TR). Cells containing any type of chromosomal aberrations were scored as "cells with aberrations" (CAB).

DNA isolation and PCR analysis

The peripheral blood samples (5-10 ml, obtained by venipuncture) were collected into heparinized Vacutainer tubes and stored at -20°C. DNA extraction was conducted using standard Chelex protocol as described in Walsh et al. (1991). PCR-based genotyping was performed for genes encoding IL-10 (-1082 G > A), IL-10 (-819/-592 C > T), IL-6 (-174 G > C), TNFα (-308 G/A), TGFβ$_1$ (codon 10 T>C), and TGFβ$_1$ codon 25 (G > C). Polymorphisms were determined by ARMS-PCR methodology, using primers described in Perrey et al. (1999) and Zakharyan et al. (2012) (Table 1). PCR reactions were performed in a 25 μl volume containing ≈ 10 ng DNA (template), with a final concentration of 1X Reaction Buffer, 1.5 mM $MgCl_2$, 5% DMSO, 250 μM dNTPs, 0.5 μM of each primer, and 1 U of Taq DNA polymerase/sample (Fischer Scientific, Pittsburgh, PA). Cycles were set as follows: 35 cycles, 1 min at 95°C, 1 min at 60°C, 1 min at 72°C, and a final extension step 10 min at 72°C. Amplification products were detected by ethidium bromide staining after 3% agarose gel electrophoresis. Randomly-selected samples (n = 15; 30% of total number) were analyzed twice to check for confidence of genotyping and in each case, complete concordance was obtained.

Statistical analysis

Statistical analysis was performed using an SPSS statistical package program (v21.0, SPSS, Chicago, IL). Allele and genotype frequencies of the four SNP were calculated using GENEPOP v. 1.2. To test the Hardy-Weinberg equilibrium (HWE), observed and expected frequencies were compared using the chi-square (χ^2) test, with a 95% confidence interval. A non-parametric Wilcoxon test was used to compare the mean frequencies of SCE and CA between groups. Multiple regression analysis was used to evaluate the influence of age and exposure years on SCE and CA frequencies of both groups. All p-values were two tailed and the level of statistical significance was set at $p < 0.05$ for all tests.

Results

Study populations

No significant differences were found between pathologists and controls in terms of mean age ($p = 0.277$) and mean years of employment ($p = 0.306$).

SCE assays

Results of the SCE assays are shown in Table 3. Significant differences were found between pathologists and controls in terms of SCE frequency ($p < 0.001$) and RI values ($p < 0.001$). In both groups, the SCE frequency did not correlate neither with years of occupational exposure ($p = 0.366$, $R = 0.130$ and $p = 0.584$, $R = 0.167$ for pathologists and controls, respectively) nor with age ($p = 0.523$, $R = 0.092$ and $p = 0.225$, $R = 0.372$ for pathologists and controls, respectively). Similarly, no gender differences were found in both groups in terms of SCE frequency ($p = 0.249$ and $p = 0.067$ for pathologists and controls, respectively). Additional outcomes from the multi-regression analyses for this parameter (including β-coefficients and 95% confidence intervals) can be found in Supplemental Table 1.

Cells with ≥ 10 SCE were defined as high frequency cells (HFC) according to Carrano and Moore (1982). Individuals for whom > 6 cells were detected that contained more than 10 SCE were classified as high frequency individuals (HFI). Ten subjects were identified as HFI among exposed and three in the control group. The HFI showed a significantly higher value of SCE with respect to Non-HFI only in the exposed group ($p = 0.005$). In the control group, although an apparent difference exists in SCE frequency between HFI and Non-HFI, this difference is not statistically significant ($p = 0.109$). Similarly, no significant differences in terms of SCE frequency were found between Non-HFI-pathologists and Non-HFI-controls ($p = 0.077$) and between HFI-pathologists and HFI-controls ($p = 0.285$).

CA assays

Results of the CA assays are shown in Table 4. This study found five types of aberrations: chromatid breaks, chromosome breaks, dicentrics, acentric fragments, tri-radial and rings (these last only in the control group). The most frequent types of aberration were chromatid breaks and chromosome breaks. Significant differences were observed between pathologists and controls in the frequency of CA/NSM and CAB/NSM ($p < 0.001$). In both groups, the level of chromosomal damage did not correlate neither with years of employment ($p = 0.094$, $R = 0.240$ and $p = 0.895$, $R = 0.045$ for pathologists and controls, respectively), nor with age ($p = 0.171$, $R = 0.197$ and $p = 0.298$, $R = 0.356$, for pathologists and controls, respectively). Additional outcomes from the multi-regression analyses for this parameter (including β-coefficients and 95% confidence intervals) can be found in Supplemental Table 1.

Cytokine gene polymorphisms and genomic damage

Allele and genotype frequencies of the analyzed cytokine gene polymorphisms were reported in Table 5. All loci were polymorphic and in HWE in both groups. Data about the association between cytokine gene polymorphisms and genomic damage, measured in terms of SCE and CA frequencies, were reported in Tables 6 and 7, respectively. No association was found between all analyzed cytokine gene polymorphisms and the CA frequency in both patho-logists and controls groups (Table 7). *Vice-versa*, among pathologists, homozygote individuals for the IL-6 G allele showed a significantly ($p = 0.017$) lower frequency of SCE with respect to heterozygote subjects. Similarly, for TGFβ_1 codon 10 locus, homozygote for T allele and hetero-zygote TC subjects showed a significantly ($p = 0.021$) lower frequency of SCE with respect to homozygote CC individuals. Finally, in the control group, no significant differences were found in the frequency of SCE between genotypes at all loci (Table 6).

Discussion

Several published studies have examined the relationship between some genetic polymorphisms and levels of genomic damage among workers occupationally exposed to xenobiotics. Most of these studies focused their attention on metabolic gene polymorphisms, while the influence of cytokine gene polymorphisms on the levels of genomic damage was scarcely taken into account. However, some cytokine

gene polymorphisms were found to be associated with the pre-disposition to a variety of diseases, including inflammatory and cancer diseases, that are known to be diseases directly or indirectly related to DNA damage (Higashimoto et al., 2006; Colakogullari et al., 2008; Smith and Humphries, 2008; Helmig et al., 2009; Kuningas et al., 2009; van Dyke et al., 2009; Westbrook et al., 2012). Other *in vitro* studies evidenced that some cytokines could be directly related to an increase of genomic damage. For example, human IL-2 and TNFα were found to increase SCE frequency in mammalian cells and in human peripheral blood lymphocytes, respectively (Lazutka, 1996), as well as IL-13 was found to be associated with a systemic induction of genotoxic parameters, such as oxidative DNA damage, single and double DNA strand breaks, and micronucleus formation (Chapman et al., 2014).

To our knowledge, no *in vivo* studies have been identified in the literature about the relationship between cytokine gene polymorphisms and levels of cytogenetic damage. To fill this gap, the present study investigated, in a sample of hospital workers chronically exposed to xenobiotics, the possible influence of six cytokine gene polymorphisms on the level of genomic damage. As general result, pathologists showed significantly higher frequencies of SCE and CA with respect to control subjects. These data further confirmed the evidence that had emerged in our previously-published articles and those of other authors, placing hospital workers as risk category of genotoxic damage caused by chronic exposure to xenobiotics (Fransman et al., 2004; Kopjar et al., 2009; Santovito et al., 2011, 2013, 2014). However, the lack of data on defined levels of exposure to different xenobiotics makes it impossible to draw a clear relationship between a specific xenobiotic and the genomic damage found in the pathologists group.

Higher frequencies of SCEs recorded among the HFI seemed to indicate the presence of a subset of individuals more susceptible to the genomic damage resulting from daily environmental exposure. This higher susceptibility could also reflect potential defects in DNA repair processes in these individuals (Garcia-Sagredo, 2008). These defects in DNA repair have been linked to genome instability, heritable cancers, premature ageing syndromes and neurological diseases (Rass et al., 2007). Notably, while the frequency of SCEs recorded among Non-HFI is very similar in both groups (4.613±0.114 and 4.046±0.162 for pathologists

and controls, respectively), a difference, although not significant, was found between HFI of both groups (7.532±0.226 and 6.827±0.221 for pathologists and controls, respectively).

This difference could be due to the fact that for pathologists the levels of SCEs recorded among HFI represent the result of the cumulative effect of both genomic susceptibility and occupational exposure to xenobiotics.

The significantly lower RI value observed among the pathologists could indicate that, for this category of workers, the risk was not only a genotoxic one but also a cytotoxic one. Pathologists might be exposed to chemicals throughout their use in healthcare environments and this occupational exposure may occur in different ways, such as inhalation of airborne agents, absorption through skin contact, or contact with patient body fluids. To minimize the risk of occupational exposure, several guidelines and safety recommendations were issued. Neverthe-less, despite the adoption of guidelines in health care institutions, published reports suggest that some health care workers do not follow the standards established by their employers, putting themselves at risk for mutagenicity (Ritchie et al., 1999).

Interestingly, by analyzing the effect of cytokine gene polymorphisms on the amount of genomic damage, a possible correlation was found between IL-6 and TGFβ_1 gene polymorphisms and levels of SCE. In particular, this study found that IL-6 GC and TGFβ_1 CC genotypes were associated with increased levels of SCE. Although in *in vitro* experiments, a relationship between increased levels of SCE and cytokine gene polymorphisms was found, the lack of data about *in vivo* experiments did not allow for comparisons to the present results. Moreover, cytokines represent a complex network where some cytokines activate or inactivate production of other cytokines. Thus, it is unclear whether the hypothetical genomic effects of cytokine gene polymorphisms were due to direct actions of a single cytokine or due to interactions with the cytokine network.

Still, in analyzing the functions of the studied cytokines, some assumptions could be made. IL-6 and TGFβ_1 are multi-functional cytokines that play an important role in proliferation and differentiation processes and in the activity of several types of cells; they also have a crucial role in acute phase responses

and in inflammatory processes pathways (Awad et al., 1998; Fishman et al., 1998; Holweg et al., 2001; Xaubet et al., 2003). The G > C single nucleotide polymorphism at the promoter position -174 of the *IL-6* gene is associated with different IL-6 plasma levels in healthy subjects (Xing et al., 1998; Terry et al., 2000). The change from G to C at position -174 creates a potential binding site for the transcription factor NF-1, thus repressing gene expression. Therefore, the C allele is associated with lower plasmatic levels of IL-6 (Fishman et al., 1998). Similarly, the T > C polymorphism on codon 10 results in a change in amino acid sequence that alters plasma levels of TGFβ_1. As a consequence, the homozygous CC genotype at codon 10 is strongly associated with lower production of this cytokine (Awad et al., 1998).

Westbrook et al. (2012) demonstrated that systemic DNA damage to peripheral leukocytes and various tissues was related to chronic intestinal inflammation in mice. Some environ-mental xenobiotics, like lead, are known to induce expression of cytokines associated with inflammatory response in occupationally exposed groups (Yucesoy et al., 1997; Mishra et al., 2003). In general, inflammatory conditions have been associated with cellular transformation and malignancy by mechanisms including induction of DNA damage and chromosomal abnormal-ities by pro-inflammatory cytokines (Higashimoto et al., 2006).

In this scenario, one could speculate that higher circulating levels of a pro-inflammatory cytokine like IL-6 could be directly associated with a longer duration/greater intensity of the inflammatory processes, and indirectly with significantly higher levels of genomic damage, as was observed among the IL-6 GG genotypes. On the other hand, it is known that TGFβ_1 is a multi-functional cytokine acting also as immunosuppressant to inhibit expression of several pro-inflammatory cytokines, including IL-6 and TNFα (Suzumura et al., 1993; Benveniste et al., 1994). In particular, TGFβ_1 can reduce macrophage production of IL-6 (Guarnizo-Zuccardi et al., 2007). Also in this case, it could be speculated that the lower levels of TGFβ_1 noted among the T/C and C/C genotypes (Awad et al., 1998) could be associated with a less efficient immuno-suppressive activity. A consequent longer duration and/or greater intensity of inflammatory processes could indirectly correlate with higher levels of genomic damage, as was observed here. Our hypothesis was corroborated by the fact that a possible role for pro-inflammatory cytokines, such as

TNFα and IL-1β, in DNA damage and in the regulation of chromosomes stability has already been described in cultured cells (Aggarwal et al., 1993; Nathan et al., 2000; Wheelhouse et al., 2003; Seidelin et al., 2005).

Nevertheless, it should be emphasized that, although the population size is adequate for the assessment of chromosomal damage, it results underpowered for SNPs analysis. In this sense our hypothesis requires further larger-scale investigations in order to be confirmed.

Conclusion

The present study found increased levels of SCE and CA in a sample of hospital pathologists compared to in control subjects. When results were analyzed according to some cytokine gene polymorphisms, an association was found between *IL-6* and *TGFβ_1* gene polymorphisms and the level of genomic damage. To our knowledge, this is the first *in vivo* study reporting a relationship between cytokine gene polymorphisms and genomic damage. Further larger-scale studies are necessary to confirm these results and to explore possible ethnic differences in the roles played by these polymorphisms on the genomic-damage risk.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content of this manuscript.

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Table 1. Demographic characteristics of studied groups.

Characteristics	Pathologists	Controls
Subjects	**50**	**50**
Sex		
Males	23	23
Females	27	27
Age		
Total sample		
Mean years [±SD]	41.12 ± 8.08	39.86 ± 8.06
Range (years)	25–60	24–66
Males		
Mean years [±SD]	44.87 ± 8.35	41.09 ± 1.86
Range (years)	28–60	27–66
Females		
Mean years [±SD]	39.56 ± 7.36	38.18 ± 1.37
Range (years)	25–52	24–53
Years of employment		
Total sample		
Mean years [±SD]	10.36 ±7.40	11.34 ± 6.34
Range (years)	1–27	1–31
Males		
Mean years [±SD]	13.39 ± 7.61	12.74 ± 7.82
Range (years)	2–24	2–31
Females		
Mean years [±SD]	7.78 ± 6.26	10.15 ± 4.56
Range (years)	1–27	1–20

Table 2. Primers and annealing temperatures for cytokines gene polymorphisms analyzed in the present study.

dbSNP ID	Cytokine	Sequence	T (°C)	PCR product size (bp)	Reference
rs909253	TNFα (-308, G>A)	5′-TCTCGGTTTCTTCTCCATCG-3′	60	184	Perrey et al. (1999)
	• Antisense primer	5′-ATAGGTTTTGAGGGGCATGG-3′			
	• G-sense primer	5-AATAGGTTTTGAGGGGCATGA-3′			
	• A-sense primer				
rs1800896	IL 10 -1082 (G> A)	5′-AGTGCCAACTGAGAATTTGG-3′	60	258	Perrey et al. (1999)
	• Antisense primer	5′-CTACTAAGGCTTCTTTGGGAG-3′			
	• G-sense primer	5′-ACTACTAAGGCTTCTTTGGGAA-3′			
	• A-sense primer				
rs1800871	IL-10 (-819, C> T)	5′-AGGATGTGTTCCAGGCTCCT-3′	60	233	Perrey et al. (1999)
	• Antisense primer	5′-CCCTTGTACAGGTGATGTAAC-3′			
	• C-sense primer	5′-ACCCFFGTACAGGTGATGTAAT-3′			
	• T-sense primer				
rs1800470	TGFβ$_1$ (Codon 25, G>C)	5′-GGCTCCCGGTTCTGCACTC-3′	60	233	Perrey et al. (1999)
	• Antisense primer	5′-GTCTGACGCCTGGCCG-3′			
	• G-sense primer	5′-GTGCTGACGCCTGGCCC-3′			
	• C-sense primer				
rs1800471	TGFβ$_1$ (Codon 10, C>T)	5′-TCCGTGGGATACTGAGACAC-3′	60	241	Perrey et al. (1999)
	• Antisense primer	5′-GCAGCGGTAGCAGCAGCG-3′			
	• C-sense primer	5′-AGCAGCGGTAGCAGCAGCA-3′			
	• T-sense primer				
rs1800795	IL-6 (-174, G>C)	5′-TCGTGCATGACTTCAGCTTTA-3′	60	190	Zakharyan et al. (2012)
	• Antisense primer	5′-AATGTGACGTCCTTTAGCATG-3′			
	• G-sense primer	5′-AATGTGACGTCCTTTAGCATC-3′			
	• C-sense primer				
	Internal Control 1	5′-GCCTTCCCAACCATTCCCTTA-3′	60	429	Perrey et al. (1999)
	Internal Control 2	5′-TCACGGATTTCTGTTGTGTTTC-3′			

Table 3. Frequency of SCE and RI values in metaphases of lymphocytes from studied subjects.

Groups	N	NSM	SCE	SCE/NSM [± SE]	M_1	M_2	M_3	RI [± SE]
Pathologists	**50**	**2500**	**12991**	**5.196 ± 0.195**[a]	**2245**	**1857**	**807**	**1.703 ± 0.051**[b]
Non-HHI	40	2000	9225	4.613 ± 0.114[c]	1759	1515	632	1.671 ± 0.069
HFI	10	500	3766	7.532 ± 0.226[c]	486	342	175	1.695 ± 0.087
Males	23	1150	6027	5.241 ± 0.250	975	888	393	1.709 ± 0.086
Females	27	1350	6984	5.159 ± 0.296	1270	969	414	1.648 ± 0.077
Controls	**50**	**2500**	**10531**	**4.212 ± 0.179**[a]	**1643**	**1972**	**1373**	**1.941 ± 0.027**[b]
Non-HHI	47	2350	9507	4.046 ± 0.162	1520	1852	1328	1.959 ± 0.026
HFI	3	150	1024	6.827 ± 0.221	123	120	45	1.660 ± 0.090
Males	23	1150	5139	4.469 ± 0.238	804	901	596	1.910 ± 0.030
Females	27	1350	5392	3.994 ± 0.260	839	1071	777	1.967 ± 0.042

[a,b] $p < 0.001$.
[c] $p = 0.005$.
N = Number of analyzed subjects; NSM = Number of scored metaphases; SCE = Sister chromatid exchanges; Metaphases; RI (Replication Index) = $(M_1 + 2M_2 + 3M_3)/N$, where M_1, M_2 and M_3 represent number of cells undergoing first, second, and third mitosis and N is total number of metaphase scored.

Table 4. Frequencies of chromosomal aberrations (CA) and cells with aberrations (CAB) in metaphases of lymphocytes from subjects.

			CAs								CAs/NSM %	CAB/NSM %
Groups	N	NSM	B′	B″	Dic	AF	R	TR	Total CA	Total CAB	Mean [± SE]	Mean [± SE]
Pathologists	**50**	**10000**	**110**	**25**	**5**	**18**	**0**	**1**	**159**	**150**	**0.0159 ± 0.002**[a]	**0.0150 ± 0.002**[b]
Males	23	4600	61	11	0	11	0	0	83	76	0.0180 ± 0.003	0.0165 ± 0.002
Females	27	5400	49	14	5	7	0	1	76	74	0.0141 ± 0.002	0.0137 ± 0.002
Controls	**50**	**10000**	**45**	**9**	**4**	**14**	**1**	**4**	**77**	**76**	**0.008 ± 0.001**[a]	**0.008 ± 0.001**[b]
Males	23	4600	11	5	1	5	1	2	25	24	0.005 ± 0.001	0.005 ± 0.001
Females	27	5400	34	4	3	9	0	2	52	52	0.010 ± 0.001	0.010 ± 0.001

[a,b] $p < 0.001$.
N = Number of analyzed subjects; NSM = Number of Scored Metaphases; B′: chromatid breaks; B″: chromosome breaks; Dic = Dicentric chromosome; AF = Acentric Fragments; R = Rings; TR = Tri- or Tetra-Radials; CAs = Chromosome Aberrations; CAB = cells with aberrations.

Table 5. Allele and genotype frequencies of cytokine gene polymorphisms among pathologists and control subjects.

	Pathologists				Controls			
Genotypes	Observed Genotypes (Frequency)	Expected Genotypes (Frequency)	χ^{2a}_{HWE} df = 1	Alleles (frequency)	Observed Genotypes (Frequency)	Expected Genotypes (Frequency)	χ^{2a}_{HWE} df = 1	Alleles (frequency)
TNF-α −308								
G/G	29 (0.580)	31.21 (0.624)	0.156	G = 79 (0.790)	33 (0.660)	34.45 (0.689)	0.061	G = 83 (0.830)
G/A	21 (0.420)	16.56 (0.332)	1.172	A = 21 (0.2170)	17 (0.340)	14.11 (0.282)	0.592	A = 17 (0.170)
A/A	0 (0.000)	2.21 (0.044)	2.205		0 (0.000)	1.45 (0.029)	1.445	
Total			3.533				2.098	
IL 10 −1082								
G/G	35 (0.700)	35.38 (0.706)	0.002	G = 84 (0.840)	29 (0.580)	29.65 (0.593)	0.014	G = 77 (0.770)
G/A	14 (0.280)	13.44 (0.269)	0.023	A = 16 (0.160)	19 (0.380)	17.71 (0.354)	0.094	A = 23 (0.230)
AA	1 (0.020)	1.28 (0.026)	0.061		2 (0.040)	2.65 (0.053)	0.157	
Total			0.087				0.265	
IL-10 (−8191)								
C/C	37 (0.740)	36.98 (0.740)	0.000	G = 86 (0.860)	40 (0.800)	40.50 (0.810)	0.006	C = 90 (0.900)
C/T	12 (0.240)	12.04 (0.241)	0.000	A = 14 (0.140)	10 (0.200)	9 (0.180)	0.111	T = 10 (0.100)
T/T	1 (0.020)	0.98 (0.020)	0.000		0 (0.000)	0.50 (0.010)	0.500	
Total			0.000				0.734	
TGF-β_1 Codon 25								
G/G	41 (0.820)	41.41 (0.828)	0.004	G = 91 (0.910)	38 (0.760)	38.72 (0.775)	0.013	G = 88 (0.880)
G/C	9 (0.180)	8.19 (0.164)	0.080	C = 9 (0.090)	12 (0.240)	10.56 (0.211)	0.196	C= 12 (0.120)
C/C	0 (0.000)	0.41 (0.008)	0.405		0 (0.000)	0.72 (0.014)	0.720	
Total			0.489				0.930	
TGF-β_1 Codon 10								
C/C	9 (0.180)	11.05 (0.221)	0.379	C = 47 (0.470)	13 (0.260)	13.01 (0.260)	0.000	C = 51 (0.510)
C/T	29 (0.580)	24.91 (0.498)	0.672	T = 53 (0.530)	25 (0.500)	24.99 (0.500)	0.000	T = 49 (0.490)
T/T	12 (0.240)	14.05 (0.281)	0.298		12 (0.240)	12.01 (0.240)	0.000	
Total			1.348				0.000	
IL-6 (−174 G>C)								
G/G	34 (0.680)	35.28 (0.706)	0.046	C = 84 (0.840)	32 (0.640)	33.62 (0.672)	0.078	C = 82 (0.820)
G/C	16 (0.320)	13.44 (0.269)	0.488	T = 16 (0.160)	18 (0.360)	14.76 (0.295)	0.711	T = 18 (0.180)
C/C	0 (0.000)	1.28 (0.026)	1.280		0 (0.000)	1.62 (0.032)	1.620	
Total			1.814				2.409	

[a]Chi-square values calculated from number of observed/expected genotypes; HWE = Hardy–Weinberg Equilibrium; df = degree freedom.

Table 6. Frequency of SCE and RI values in metaphases of lymphocytes from subjects – according to cytokine polymorphism.

Pathologists	N	NSM	SCEs	SCEs/NSM [± SE]	M_1	M_2	M_3	RI [± SE]
IL10 (−1082)								
(GG)	35	1750	9357	5.347 ± 0.244	1561	1290	536	1.643 ± 0.069
(GA)	14	700	3371	4.816 ± 0.328	613	540	269	1.786 ± 0.106
(AA)	1	50	263	5.260	71	27	2	1.310
IL10 (−819)								
(CC)	37	1850	10114	5.467 ± 0.242	1583	1414	646	1.752 ± 0.057
(CT)	12	600	2637	4.395 ± 0.177	618	417	131	1.538 ± 0.109
(TT)	1	50	240	4.80	44	26	30	1.860
IL6								
(GG)	34	1700	8806	5.180 ± 0.250[a]	1538	1203	546	1.681 ± 0.064
(GC)	16	800	4185	5.231 ± 0.307[a]	707	654	261	1.749 ± 0.083
TNF								
(GG)	29	1450	7623	5.257 ± 0.284	1266	1063	567	1.756 ± 0.065
(GA)	21	1050	5368	5.112 ± 0.225	979	794	240	1.565 ± 0.088
TGFβ1 codon 10								
(TT)	12	600	3104	5.173 ± 0.493[b]	447	366	318	1.889 ± 0.132
(TC)	29	1450	7508	5.178 ± 0.256[c]	1372	1137	120	1.639 ± 0.057
(CC)	9	450	2379	5.287 ± 0.328[b,c]	426	354	120	1.660 ± 0.105
TGFβ1 codon 25								
(GG)	41	2050	10655	5.198 ± 0.227	1902	1525	592	1.641 ± 0.060
(GC)	9	450	2336	5.191 ± 0.305	343	332	215	1.836 ± 0.152
IL10 (−1082)								
(GG)	29	1450	6271	4.325 ± 0.254	912	1173	801	1.952 ± 0.034
(GA)	19	950	3792	3.992 ± 0.251	658	725	518	1.927 ± 0.042
(AA)	2	100	493	4.930 ± 1.470	77	76	48	1.865 ± 0.295
IL10 (−819)								
(CC)	40	2000	8482	4.241 ± 0.203	1326	1589	072	1.930 ± 0.029
(CT)	10	500	2074	4.148 ± 0.419	321	385	295	1.976 ± 0.067
IL6								
(GG)	32	1600	6623	4.139 ± 0.237	1037	1301	848	1.932 ± 0.028
(GC)	18	900	4056	4.507 ± 0.323	595	679	533	1.972 ± 0.051
TNF								
(GG)	33	1650	6647	4.028 ± 0.201	1097	1267	937	1.952 ± 0.033
(GA)	17	850	3909	4.599 ± 0.334	550	707	430	1.914 ± 0.042
TGFβ1 codon 10								
(CC)	13	650	2838	4.366 ± 0.358	496	489	316	1.863 ± 0.064
(CT)	25	1250	5337	4.270 ± 0.253	784	999	717	1.973 ± 0.030
(TT)	12	600	2381	3.968 ± 0.396	367	486	334	1.951 ± 0.058
IL10 (−8191)								
(CC)	40	2000	8482	4.241 ± 0.203	1326	1589	072	1.930 ± 0.029
(CT)	10	500	2074	4.148 ± 0.419	321	385	295	1.976 ± 0.067

[a] $P = 0.017$.
[b,c] $P = 0.021$.

Table 7. Frequencies of chromosomal aberrations (CAs) and cells with aberrations (CAB) in lymphocytes of subjects – according to cytokine polymorphism.

Pathologists	N	NSM	B′	B″	Dic	AF	R	TR	Total CAs	Total CAB	CAs/NSM Mean [± SE]	CAB/NSM Mean [± SE]
IL10 (−1082)												
(GG)	35	7000	78	20	3	13	0	1	115	109	0.0164 ± 0.002	0.0156 ± 0.002
(GA)	14	2800	30	5	2	5	0	0	42	39	0.0150 ± 0.002	0.0147 ± 0.002
(AA)	1	200	2	0	0	0	0	0	2	2	0.010	0.010
IL10 (−819)												
(CC)	37	7400	83	19	2	15	0	1	120	111	0.0162 ± 0.002	0.0150 ± 0.002
(CT)	12	2400	19	5	3	2	0	0	29	29	0.0121 ± 0.002	0.0121 ± 0.002
(TT)	1	200	8	1	0	1	0	0	10	10	0.050	0.050
IL6												
(GG)	34	6800	70	19	3	16	0	1	109	102	0.0160 ± 0.002	0.0150 ± 0.002
(GC)	16	3200	40	6	2	2	0	0	50	48	0.0156 ± 0.003	0.0150 ± 0.003
TNF												
(GG)	29	5800	65	16	2	10	0	0	93	88	0.0160 ± 0.002	0.0152 ± 0.002
(GA)	21	4200	45	9	3	8	0	1	66	62	0.0157 ± 0.002	0.0148 ± 0.002
TGFβ1 codon 10												
(TT)	12	2400	31	4	1	6	0	0	42	37	0.0175 ± 0.004	0.0154 ± 0.003
(TC)	29	5800	60	15	4	10	0	1	90	86	0.0155 ± 0.002	0.0148 ± 0.002
(CC)	9	1800	19	6	0	2	0	0	27	27	0.0150 ± 0.003	0.0139 ± 0.002
TGFβ1 codon 25												
(GG)	41	8200	86	15	5	14	0	0	120	114	0.0146 ± 0.002	0.0139 ± 0.001
(GC)	9	1800	24	10	0	4	0	1	39	36	0.0217 ± 0.006	0.0200 ± 0.005
IL10 (−1082)												
(GG)	35	7000	78	20	3	13	0	1	115	1089	0.0164 ± 0.002	0.0156 ± 0.002
(GA)	14	3800	19	4	2	1	1	1	28	28	0.007 ± 0.001	0.007 ± 0.001
(AA)	2	400	2	0	1	0	0	1	4	4	0.010 ± 0.004	0.010 ± 0.004
IL10 (−819)												
(CC)	40	8000	35	7	3	12	0	2	59	58	0.007 ± 0.001	0.007 ± 0.001
(CT)	10	2000	10	2	1	2	1	2	18	18	0.009 ± 0.001	0.009 ± 0.001
IL6												
(GG)	32	6400	35	5	2	9	0	3	54	54	0.008 ± 0.001	0.008 ± 0.001
(GC)	18	3600	12	3	2	5	1	1	24	24	0.007 ± 0.002	0.007 ± 0.002
TNF												
(GG)	33	6600	29	5	4	6	1	0	49	48	0.007 ± 0.001	0.007 ± 0.001
(GA)	17	3400	16	4	0	8	0	0	28	28	0.008 ± 0.001	0.008 ± 0.001
TGFβ1 codon 10												
(CC)	13	2600	9	3	2	4	0	0	18	17	0.007 ± 0.002	0.007 ± 0.002
(CT)	25	5000	27	3	1	8	0	4	43	43	0.009 ± 0.001	0.009 ± 0.001
(TT)	12	2400	9	3	1	2	1	0	16	16	0.007 ± 0.002	0.007 ± 0.002
TGFβ1 codon 25												
(GG)	38	7600	37	6	1	11	0	3	58	57	0.008 ± 0.001	0.008 ± 0.001
(GC)	12	2400	8	3	3	3	1	1	19	19	0.008 ± 0.002	0.008 ± 0.002

N = Number of analyzed subjects; NSM = Number of scored metaphases; B′: chromatid breaks; B″: chromosome breaks; Dic = Dicentric chromosome; AF = Acentric fragments; R = Rings; TR = tri- or tetra-radials; CAs = Chromosome aberrations; CAB = cells with aberrations.