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Evaluation of Chromosome Aberrations in Subjects Exposed to Environmental Tobacco Smoke in Tamilnadu, India

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Abstract Numerous expert panels have concluded that there is sufficient evidence to classify involuntary smoking (or passive smoking) as carcinogenic to humans. The aim of this study is to establish whether passive smoking increases the frequency of chromosomal aberrations (CA) in peripheral blood lymphocytes compared to controls in Tamil Nadu, India. In the present study, CA increased with an increase in environmental tobacco smoke (ETS) and active smoke exposure period in passive smokers quantified on the basis of serum cotinine levels. The passive subjects were compared with healthy normal controls to validate the results. In conclusion, these data are compatible with the current knowledge on the mechanisms of carcinogenesis of tobacco-related cancers, occurring not only in active smokers but with a high biological plausibility also in passive smokers.

Keywords Passive smoking · Chromosomal aberrations · Serum cotinine

It has been estimated that the annual mortality associated with cigarette smoking was 3 million in 1990, 4 million in 1998, and is expected to rise up to 8.4 million in 2020 (WHO 2002). In India, the smoking habits in lower and middle socio-economic class are more common among men than women (Gupta 1996; WHO 1997). Cigarette smoking is responsible for the vast majority of lung cancers and is associated with cancers of the mouth, pharynx, larynx, oesophagus, stomach, pancreas, kidney, ureter, bladder, colon and uterine cervix. Interestingly, exhaustive report on health consequences of involuntary smoking by the United States Surgeon General (DHHS 1984) and reports by the United States Environmental Protection Agency (US EPA 1992) highlighted the increased risks of several diseases similar to those seen among smokers, in persons exposed to passive smoking or environmental tobacco smoking (ETS) at home or at work place. Passive smoke exposure is a cause of cardiovascular disease, cancer, and respiratory disease (DHHS 2006; Hozawa et al. 2006).

Most of the chemicals found in cigarette smoke are genotoxic (IARC 1986), and therefore, chromosome damage appears to be an excellent biomarker for determining the effect of exposure to smoking. The chromosomal aberrations (CA) assay is important for monitoring the populations exposed to genotoxic agents because it allows the evaluation of the entire genome to identify mutagenic and carcinogenic chemicals (Au et al. 1998). Therefore, the analysis of CA in peripheral blood lymphocytes can be used as a biomarker of health outcome, measuring genetic damage due to exposure that results from non-repaired primary lesions. Numerous studies have been conducted using the CA assay to monitor the effect of cigarette smoking but the results are inconsistent. While a large population study indicated that CA frequencies in lymphocytes were not increased by smoking (Bender et al 1988) another showed that smoking caused a 10–20% increase (NST 1990).

The spouse, child and close associates face the consequence without being directly involved in active smoking. One of the very difficult tasks in most studies on passive smoking is to quantify an ETS and active smoke exposure. The number of cigarettes was the best measure of exposure to husbands' smoking, while exposure at workplace was more

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strongly related to the duration of exposure (Riboli et al. 1990). It was also shown that the potential bias due to smoker misclassification was very unlikely to be responsible for the increased health risks observed in the epidemiological studies on ETS (Riboli et al. 1995). Also, in the present study serum cotinine levels were measured in both passive and control subjects because it has been used as a biomarker of tobacco smoke to validate current smoking habit (Schluter et al. 2002; Kaufman et al. 2002). Among the available biochemical measures, cotinine, a major metabolite of nicotine, is considered to be the most reliable and valid biochemical marker of active nicotine consumption and exposure to active smoke and ETS because of its high sensitivity and specificity (Klebanoff et al. 2001; NCEH 2003). Hence in the present investigation, both passive and control subjects were quantified based on the duration of the exposure after estimating the serum cotinine level.

The aim of this investigation was to establish whether ETS and active cigarette smoke increases the frequency of CA in peripheral blood lymphocytes of passive smokers. This was compared and correlated with results obtained from control subjects.

Materials and Methods

Using an initial short questionnaire in self-report format and on the basis of the serum cotinine levels and, individuals were selected as passive smokers (n = 43) and controls (n = 43). The essential inclusion criteria for all were being ≥20 years of age and minimum of 8-years of exposure. The passive subjects were selected based on both exposure period and in an age wise manner with controls subjects matching them only on the basis of age. They were grouped as follows; Group I ≤6 h of exposure/day and below 30 years of age and Group II ≥6 h of exposure/day and above 31 years of age.

Experimental subjects were asked whether they were regularly exposed to ETS at home, work (including on the way to and back from work), respectively. The duration of ETS and active smoke exposure at home, work, was recorded and used to group the passive smokers. Passive smokers selected were mostly wives and children of active smokers along with non-smoking owners and workers of teashops provided with smoking facility. The adult subjects were non-smokers, lived and worked in the monitored areas. Combined use of appropriately worded self-reported questionnaires and cotinine levels were used to estimate ETS and active cigarette smoke exposure. Also the cotinine levels were checked in a weekly manner for 6 months before quantifying the passive subjects.

Controls were healthy volunteers; never smokers; and had minimal or no contact with smokers. All passive subjects were recruited sequentially, with controls being matched to the respective subjects in terms of age (±2 years relaxed). Venous blood samples (5 mL) were drawn in heparinized syringes from each subject. The work was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Cotinine was analysed using the enzyme-linked immunosorbent assay (ELISA) kit (STC, USA). A cotinine level of 15 ng/mL has been suggested as a cut-off for active smoking (Klebanoff et al. 2001; Kaufman et al. 2002) and was used in the present study to divide subjects into passive smokers (<15 ng/mL) and controls. Serum cotinine levels have been divided into five categories for most analyses. The lowest category represents undetectable levels [coded as 0.025 ng/mL (0.14 nmol/L)]. The next two categories [0.050–0.500 and 0.501–15.00 ng/mL (0.28–2.84 and 2.85–85 nmol/L)] represent levels typically found among non-smokers exposed to environmental tobacco smoke (ETS).

All chemical reagents were purchased from Sigma Chemicals, except colcemid that was obtained from Gibco Laboratory. Blood samples were set up to establish leukocyte cultures following standard procedures in our laboratory (Hoyos et al. 1996). About 0.5 mL blood was added to 4.5 mL RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 1% streptomycin-penicillin, 0.2 mL reagent grade phytohemagglutinin, and was incubated at 37°C. After 50 h, cultures were treated with 0.1 mg/mL colcemid to arrest the cells at metaphase in mitosis. Lymphocytes were harvested after 52 h by centrifuging cell suspension to remove culture medium (800–1,000 rpm), addition of hypotonic solution (KCl 0.075 M) at 37°C for 20 min to swell the cells, and treated twice with Carnoy's fixative (3:1 ratio of methanol/acetic acid). Slides were carefully dried on a hot plate (56°C, 2 min). Three days later, slides were stained using the Giemsa stain. For the CA analysis, 50 complete metaphase cells in first cell cycle were evaluated per subject under a Leica microscope (100×) to identify numerical and structural CA. The collected data were registered on master tables and later transferred to a computer file.

All statistical analyses were performed using software SPSS for Windows, version 13. To assess the Group statistics of passive smokers and controls for mean age and CA, mean ± SD was used. Finally confirmed by using the ANOVA test through within and between groups of passive subjects and controls.

Results and Discussion

A total of 86 subjects, corresponding to 43 passive smokers, and 43 controls were recruited for this study. In the

present study, passive smokers 32.5% males and 67.5% females were selected, whereas in control groups 21% males and 79% females were selected.

With regard to serum cotinine levels, group I passive subjects showed maximum value of 6.8 and minimum value was recorded as 2.7 and in Group II, it was 11.7 and 3.5, respectively.

Group statistics which was based on equal division of passive and control subjects into below 6 h of exposure and above 6 h of exposure is as follows: In Group I, mean ± SD values in passive subjects and controls were 5.00 ± 1.68, and 1.16 ± 0.92, respectively. In Group II the values were 9.04 ± 3.73 and 2.76 ± 2.12. The ANOVA values for within and between groups of passive subjects showed significant results. In control subjects insignificant values were obtained. Chromosomal and chromatid type aberrations are as presented in Tables 1 and 2.

Tobacco smoking is the main cause of mortality worldwide. In the present study, all passive smokers were both male and female subjects and in controls too, both males and female samples were recruited. Similar study has been carried out elsewhere (Monica Sierra-Torres et al. 2004). Surveys on health effects by spousal smoking among women who had never been primarily smokers are concerned with lung cancer or heart diseases (Zhang et al. 2005). So in the present study, among passive smokers and controls, more number of female subjects have been selected when compare to male experimental subjects. Most studies on ETS exposure involved children but in the present study, exposure at workplaces also has been used to categorize the different groups of passive smokers.

In the present study, passive subjects were grouped based on exposure period, in a similar study has been carried out among white-collar active smokers and passive smokers (non- and ex-smokers) at workplace (Fracasso et al. 2006). A direct physical measurement of exposure to tobacco smoke would reduce potential problems associated with self-report, such as inaccurate recall, underreporting, and misinterpretation of questions. In the present study, serum cotinine have been selected because other markers (e.g., carbon monoxide, cyanide, nicotine-derived nitrosoamines) are non-specific, insensitive, technically demanding or have high baseline values even in non-smokers (Benowitz et al. 1999). Non-smokers exposed to typical levels of ETS have cotinine levels of less than 1 ng/mL, with heavy exposure to ETS producing levels in the1–15 ng/mL range (NCEH 2003), in the present study too, both the passive and controls subjects were selected in the above estimated range.

In this study, Cotinine levels were checked in a weekly manner for 6 months before quantifying the subjects as passive smokers. Measuring cotinine is preferred over measuring nicotine because cotinine persists longer in the body. The average half life of cotinine in different body fluids in adults is approximately 20 h, compared with a half-life of 2 h for nicotine, making it a good indicator of the integrated exposure over the previous 2–3 days (Benowitz 1999; Rebagliato 2002).

Cigarette smoking is responsible for a substantial number of human health problems (Weir et al. 2003). However, the causal relationship between passive smoking, the induction of biological effects, and the extent of the disease burden among passive smokers have not been fully documented. In the present study, higher frequency of CA was observed among passive smokers compared to controls, they being very much lower in the latter. According to the recent evaluation by the International Agency for Research on Cancer, involuntary smoking causes lung cancer in never-smokers with an excess risk in the order of 20% for women and 30% for men (IARC 2004). In the workplace, an increase of 16–19% risk of lung cancer has been found among never-smokers (IARC 2004). These observations are supported by bioassays proving the mutagenic activity of cigarette smoke chemicals (Conforti-Froes et al. 1997). Furthermore, PAHs and several *N*-nitrosamines and aromatic amines, present in passive smoke, have been found to be carcinogenic due to their capacity to react with DNA (IARC 2004). Therefore, the results of the present study suggest that the genotoxic effects in lymphocytes from passive smokers are most likely caused by cigarette smoke constituents, providing a potential mechanism for cancer development.

Few studies have been conducted to evaluate the genotoxic effects of passive smoking but results are still inconsistent. In the present study, the increase in CA frequencies over the passive smokers is much higher than those reported in the literature (Bender et al. 1988). In this context, the present study indicates that smoking can induce genetic damage not only in smokers but also in passive smokers and that they present a significant number of CA when compared to controls. Also a correlation between serum cotinine levels and CA was found in the passive smokers thus proving the efficacy of serum cotinine as an effective biomarker in measurement of cigarette smoke exposure. Thus, the results highlight the importance of collecting quantitative data on passive smoking to understand clearly the role played by chromosome damage in cancer risk. Furthermore, the above findings support the analysis of CA as a reliable biomarker for monitoring populations exposed to passive smoking and strengthen local initiatives to fund lung cancer incidence and mortality registry programs.

In the present study, CA increased with age and exposure period in experimental groups and in age wise manner in controls, though it was very low in the latter. The results of the present study also indicate a role for age in

Table 1 Frequency of CA in passive smokers

Subject#II	Age	Gender	Group	Serum cotinine level[a]	Chromatid-type aberrations[b]	Chromosome-type aberrations[c]	Total
PS1	29	M	I	3.2	2	0	2
PS2	27	F	I	3.7	3	0	3
PS3	24	M	I	4.9	3	1	4
PS4	41	F	II	6.6	5	2	7
PS5	25	F	I	4.3	4	0	4
PS6	43	F	II	6.5	6	2	8
PS7	38	F	II	4.0	3	1	4
PS8	28	M	I	4.3	3	2	5
PS9	23	M	I	3.7	4	0	4
PS10	25	F	I	6.8	7	2	9
PS11	37	F	II	6.0	5	3	8
PS12	45	F	II	3.5	2	1	3
PS13	43	M	II	3.6	3	0	3
PS14	52	F	II	9.9	11	1	12
PS15	24	M	I	4.9	3	3	6
PS16	39	M	II	3.6	2	2	4
PS17	46	F	II	9.3	9	2	11
PS18	26	F	I	3.1	3	2	5
PS19	24	M	I	3.8	4	1	5
PS20	41	F	II	5.6	6	0	6
PS21	57	F	II	6.4	7	1	8
PS22	28	F	I	5.9	5	2	7
PS23	25	M	I	3.8	4	1	5
PS24	50	F	II	6.0	5	2	7
PS25	46	F	II	6.3	7	0	7
PS26	43	F	II	10.3	10	1	11
PS27	39	M	II	6.9	7	0	7
PS28	47	F	II	8.8	8	3	11
PS29	55	F	II	11.4	9	4	13
PS30	29	F	I	2.9	4	0	4
PS31	38	M	II	7.5	7	2	9
PS32	27	F	I	4.8	3	2	5
PS33	41	F	II	10.4	9	3	12
PS34	54	F	II	11.7	12	4	16
PS35	63	F	II	10.9	11	5	16
PS36	45	F	II	9.6	11	0	11
PS37	23	M	I	5.4	6	0	6
PS38	46	F	II	11.2	13	2	15
PS39	52	F	II	7.2	7	2	9
PS40	27	M	I	2.7	3	0	3
PS41	29	F	I	4.6	4	3	7
PS42	26	M	I	4.8	5	1	6
PS43	43	F	II	5.4	7	1	8

[a] Cotinine level: passive smokers (<15 ng/mL); (PS#: passive smokers)

[b] Chromatid-type CAs: (dicentrics; chromatid gaps; chromatid breaks)

[c] Chromosome-type CAs: (break; gap; exchange)

Group I: 6 h and below exposure period and below 30 years of age

Group II: 6 h and above exposure period and above 31 years of age

(As recommended by the International standard for chromosomal nomenclature (ISCN) 50 well spread metaphases were scored for each subject)

chromosome alterations observed in peripheral blood lymphocytes as noted in the controls. An age-related increase in aneuploid cells in human lymphocytes has been reported in a number of studies (Nowinski et al. 1990).

To the best of our knowledge, this study constitutes the first scientific evidence on the genotoxic effect of passive smoking confirmed by serum cotinine level in Tamil Nadu population. In this context, this study provides important

Table 2 Frequency of CA in controls

Control#	Age	Gender	Group	Serum cotinine level[a]	Chromatid-type aberrations[b]	Chromosome-type aberrations[c]	Total
CS1	27	F	I	0.3	0	0	0
CS2	28	F	I	0.2	1	0	1
CS3	26	F	I	0.4	0	0	0
CS4	43	F	II	0.8	2	1	3
CS5	27	F	I	0.5	2	0	2
CS6	43	F	II	0.3	0	1	1
CS7	36	F	II	0.4	0	0	0
CS8	28	M	I	0.7	0	0	0
CS9	25	M	I	0.6	2	0	2
CS10	26	F	I	0.9	1	0	1
CS11	37	F	II	0.2	2	0	2
CS12	46	F	II	0.3	1	1	2
CS13	42	F	II	0.4	1	1	2
CS14	53	F	II	0.6	3	0	3
CS15	26	F	I	0.7	1	2	3
CS16	39	M	II	0.4	2	0	2
CS17	47	F	II	0.3	2	0	2
CS18	27	F	I	1.2	1	1	2
CS19	26	M	I	0.7	0	1	1
CS20	43	F	II	0.6	4	0	4
CS21	58	F	II	0.5	3	0	3
CS22	27	F	I	0.6	2	0	2
CS23	26	M	I	0.4	1	0	1
CS24	52	F	II	0.5	7	0	7
CS25	47	M	II	0.4	3	1	4
CS26	43	F	II	0.8	1	1	2
CS27	39	F	II	0.3	0	0	0
CS28	46	F	II	0.6	4	1	5
CS29	57	F	II	0.2	5	0	5
CS30	28	M	I	0.6	1	0	1
CS31	40	F	II	0.4	0	0	0
CS32	25	F	I	0.7	0	0	0
CS33	42	F	II	0.3	3	0	3
CS34	54	M	II	0.1	4	1	5
CS35	64	F	II	0.4	7	1	8
CS36	45	F	II	0.8	0	0	0
CS37	25	F	I	0.3	1	0	1
CS38	47	F	II	0.7	1	0	1
CS39	54	F	II	0.8	3	1	4
CS40	26	M	I	0.9	0	0	0
CS41	29	F	I	0.7	2	0	2
CS42	27	F	I	0.6	2	0	2
CS43	42	F	II	0.9	1	0	1

[a] Cotinine level: controls (<2 ng/mL); (CS#: control subject)

[b] Chromatid-type CAs: (dicentrics; chromatid gaps; chromatid breaks)

[c] Chromosome-type CAs: (break; gap; exchange)

Group I: below 30 years of age

Group II: above 31 years of age

(As recommended by the International standard for chromosomal nomenclature (ISCN) 50 well spread metaphases were scored for each subject)

data to support National policies to protect from the social, economical, and environmental consequences of cigarette consumption, as well as to prevent future generations from emotional and physical burden associated with cancer.

This study is also aimed at examining the possible effects of cigarette smoke in lymphocyte CA of subjects exposed to ETS and active smoke at the workplace and home. The workplace and public buildings are by far the

commonest source of exposure, and the occupational category mostly exposed was white-collar workers (Veglia et al. 2003), evidence that supported the recent promulgation of anti-smoking legislation in Italy. Results of the present study demonstrate that the passive smokers exposed to tobacco smoke in a confined ambience display significant increase in CA and hence a probable cancer risk.

In conclusion, the data reviewed are compatible with the current knowledge on the mechanisms of carcinogenesis of tobacco-related cancers, occurring not only in smokers but with a high biological plausibility also in involuntary smokers.

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