CHAPTER III

RESULTS

1. General characteristics of the study populations

The general characteristics of the exposed and control groups are summarised in Table 1. The individuals were identified in term of age, duration they had worked, alcohol consumption and smoking habits. Data of 32 petrol pump workers were collected from various gasoline stations of central Chiang Mai, Thailand. And the 30 matched controls were selected from students of the Faculty of Medicine, Chiang Mai University, who had not been currently or previously occupationally exposed to gasoline. The age distribution of petrol pump workers and control subjects were 21.1±1.74 and 20.2±0.77 years, respectively. The duration of occupational exposure to gasoline among exposed subjects (duration of work) was 3 months to 5 years (Table 1).

2. Results of chromosomal aberrations analysis

The results of structural chromosome aberrations in the control and exposed groups are shown in Tables 2, 3, 4, and 5. The total number of cells with aberrations was 32 in the control group and was 60 in the exposed group (Table 4).

Table 5 shows the results of chromosomal aberrations in the both the control and exposed groups according to types of aberrations. In this study, both chromatid type and chromosome type aberrations in the exposed group were 42 and 18, whereas in the control group were 17 and 15, respectively. There was a significant difference in total number of aberration and chromatid type aberration between the workers and the control group. For the chromosome type aberration, there was no significant difference between the two groups (Table 5).

Table 1 Relevant general characteristics of the petrol pump workers and control subjects.

Parameters		Control subjects	Exposed workers
Number of subjects		30	32
Age (mean±S.D., years)		20.2±0.77	21.1±1.74
Gender		male	male
Duration of work in petrol pump			
3 months-1 year		-	24
1 year-5 years		-	8
Alcohol consumption	Yes	2	4
	No	25	16
	Abstinent	3	12
Cigarette smokers		1	10
Non-smokers		29	22

Table 2 Shows the frequency of cell with aberrations in individual subject of the control group.

Groups	Subject No.	Number of cell with aberrations
Control (n=30)	1	1
	2	0
	3	2
	4	3
	5	3
	6	2
	7	1
	8	1
	9	0
	10	2
	11	2
	12	0
	13	1
	14	2
	15	3
	16	0
	17	2
	18	1
	19	0
	20	1
	21	0
	22	0
	23	1
	24	0
	25	1
	26	0

Table 2 Continued.

Groups	Subject No.	Number of cell with aberrations
Control (n=30)	27	2
	28	0
	29	0
	30	1

Table 3 Shows the frequency of cell with aberrations in individual subject of the exposed group.

Groups	Subject No.	Number of cell with aberrations
Exposed (n=32)	1	2
	2	0
	3	3
	4	0
	5	3
	6	4
	7	1
	8	1
	9	0
	10	2
	11	0
	12	3
	13	1
	14	1
	15	0
	16	7

Table 3 Continued.

Groups	Subject No.	Number of cell with aberrations
Exposed (n=32)	17	1
	18	2
	19	3
	20	2
	21	3
	22	2
	23	1
	24	4
	25	1
	26	2
	27	2
	28	1
	29	3
	30	2
	31	2
	32	1

Table 4 Shows frequency of structural chromosome aberrations in the exposed and control groups.

Groups	Number of subjects	Number of cell at metaphases analyzed	Total number of cell with aberrations
Controls	30	3000	32
Exposed	32	3200	60*

*Statistically significant compared with the control group (P=0.012, Chi-Square tests).

Table 5 Frequency of structural chromosome aberrations in the exposed and control groups according to types of aberrations.

	Groups		P Value
	Control	Exposed	
Total number of cell with aberrations	32	60	0.012*
Chromatid type aberrations	17	42	0.004*
chromatid gap	14	20	
chromatid break	3	22	
chromatid exchange	0	0	
Chromosome type aberrations	15	18	0.870
chromosome gap	11	8	
chromosome break	2	4	
acentric fragments	2	5	
dicentric chromosomes	0	1	
ring chromosome	0	0	
other	0	0	

*Statistically significant compared with the controls (Chi-Square tests).

Figure 9 Normal metaphase chromosomes of exposed donor prepared from lymphocyte culture. The chromosomes were stained with conventional Giemsa stains or non-banding technique (1,000X).

Figure 10 Metaphase chromosomes of exposed donor prepared from lymphocyte culture showing a chromatid break (arrow) (1,000X).

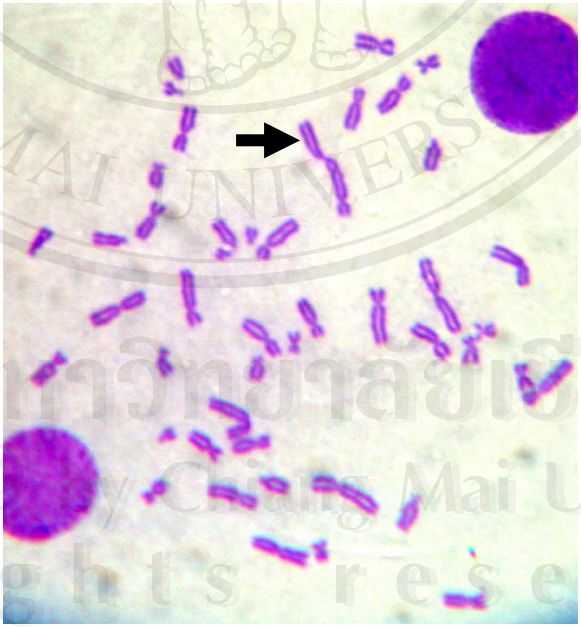

Figure 11 Metaphase chromosomes of exposed donor prepared from lymphocyte culture showing a dicentric chromosome (arrow) (1,000X).

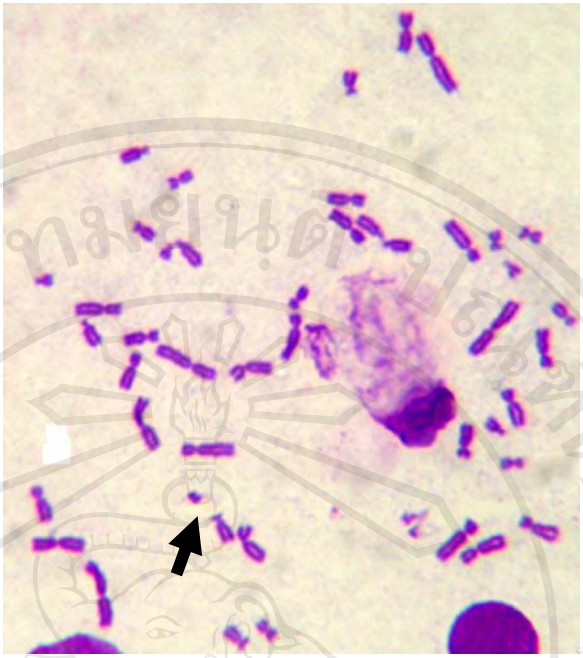

Figure 12 Metaphase chromosomes of control donor prepared from lymphocyte culture showing an acentric fragment (arrow) (1,000X).

3. Results of the comet assay

The results of the comet assay on the extent of DNA damage are presented in Table 6 and 7. The tail length and tail moment parameters were used for evaluation of DNA damage by measuring from 100 comet cells per subject.

Table 6 The mean values of tail length and tail moment in the control subjects (measured from 100 comet cells per subject).

Groups	Subject No.	Mean tail moment (μm)	Mean tail length (μm)
Control (n=30)	1	0.38	1.54
	2	0.01	0.30
	3	0.06	0.75
	4	0.65	4.28
	5	0.24	1.82
	6	0.05	0.49
	7	0.12	1.04
	8	0.06	0.88
	9	0.21	1.29
	10	0.10	0.72
	11	0.34	1.45
	12	0.15	1.09
	13	0.41	2.12
	14	1.07	4.17
	15	0.64	3.12
	16	0.15	1.13
	17	0.30	1.54
	18	1.08	3.79
	19	0.11	0.96
	20	0.09	0.83

Table 6 Continued.

Groups	Subject No.	Mean tail moment (μm)	Mean tail length (μm)
Control (n=30)	21	0.41	1.58
	22	0.77	2.89
	23	0.17	1.09
	24	0.35	1.36
	25	0.19	1.24
	26	0.21	1.27
	27	0.31	1.39
	28	0.24	1.14
	29	0.23	1.05
	30	0.14	0.93

Table 7 The mean values of tail length and tail moment in the petrol pump workers (measured from 100 comet cells per subject).

Groups	Subject No.	Mean tail moment (μm)	Mean tail length (μm)
Exposed (n=32)	1	10.49	22.82
	2	10.92	23.90
	3	1.48	5.76
	4	1.07	5.30
	5	1.83	7.44
	6	0.44	2.52
	7	0.37	1.80
	8	0.31	1.40
	9	0.13	1.22

Table 7 Continued.

Groups	Subject No.	Mean tail moment (μm)	Mean tail length (μm)
Exposed (n=32)	10	0.26	1.46
	11	0.69	3.25
	12	0.67	3.27
	13	0.57	2.47
	14	0.20	1.30
	15	0.23	1.48
	16	1.54	6.50
	17	1.28	3.67
	18	1.78	5.19
	19	3.11	8.11
	20	1.17	4.30
	21	0.62	3.51
	22	1.15	5.62
	23	1.87	6.03
	24	0.96	3.57
	25	7.26	14.88
	26	1.64	4.36
	27	2.32	6.29
	28	2.49	6.69
	29	1.72	6.84
	30	0.13	1.16
	31	0.35	2.11
	32	0.34	1.56

Table 8 The ranges and means of tail length and tail moment in the control and exposed groups.

Parameters	Groups		P value
	Control	Exposed workers	
Tail length distribution (μm)	0.30-4.28	1.16-23.90	
Tail moment distribution (μm)	0.01-1.08	0.13-10.92	
Mean tail length (μm) (mean±S.D.)	1.57±1.03	5.51±5.46	0.000*
Mean tail moment (μm) (mean±S.D.)	0.31±0.27	1.85±2.67	0.000*

*Statistically significant compared with the controls (P=0.000, Mann-Whitney U-test).

Tables 6 and 7 show the distribution of tail length and tail moment values, determined in 100 comet cells/subject, of the two groups. Distribution of both comet parameters appeared to be wider in exposed group compared with the controls. As for the control group, values of tail lengths ranged from 0.3 to 4.28 μm, whereas the values of tail moments ranged from 0.01 to 1.08 μm. In the exposed group, values of tail lengths of the comet cells varied between 1.16 and 23.90 μm. The tail moment values varied between 0.13 and 10.92 μm.

In the Table 8, the mean value of tail length in the control group was 1.57±1.03 μm, whereas in the exposed group was 5.51±5.46 μm. And the mean value of tail moment for control subjects was 0.31±0.27 μm and 1.85±2.67 μm in exposed workers. The comparison between control and exposed groups shows that the levels of DNA damage in terms of tail length and tail moment were significantly increased in the exposed workers (P=0.000, Mann-Whitney U-test).

Figure 13 A normal cell of control donor embedded in agarose gel with undetectable DNA fragmentation and no comet tail.

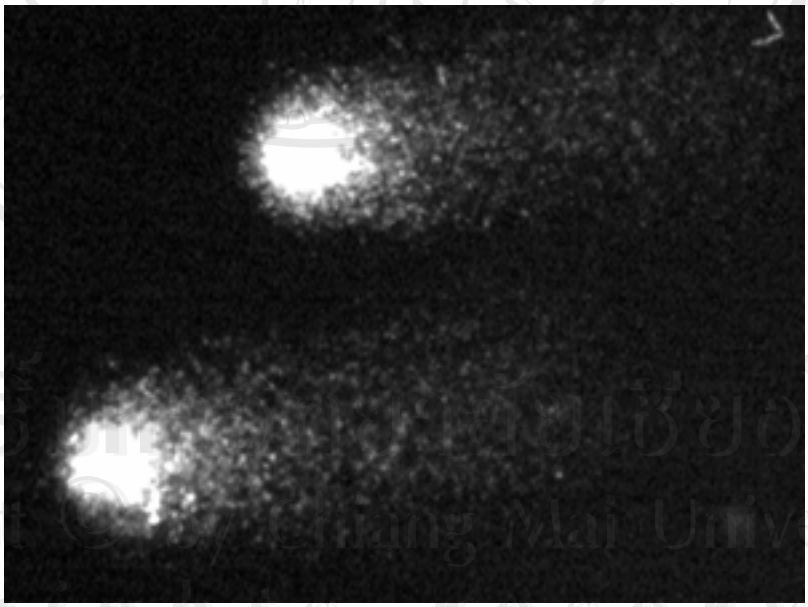

Figure 14 Two comet cells of exposed donor embedded in agarose gel showing fragmentation of DNA, evidenced by migration of DNA out of the comet head and the comet tails detected.

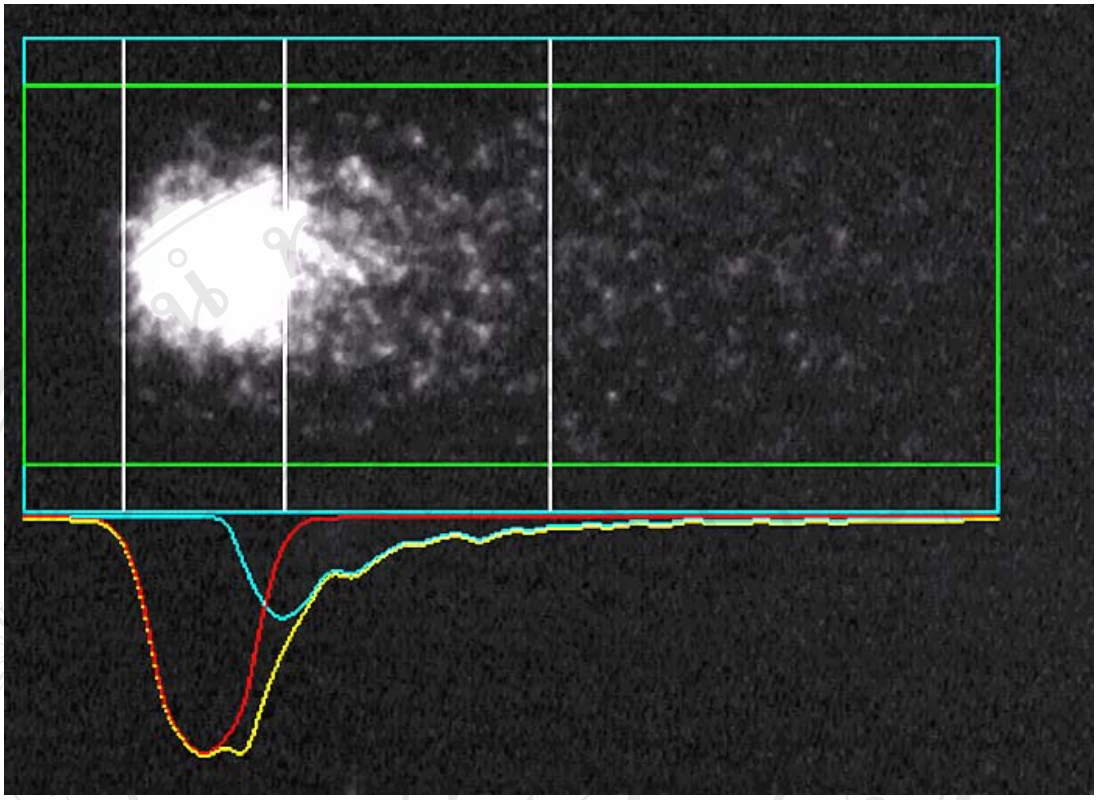

Figure 15 Measurement of comet cell by a computerized image analysis system (Comet Imager, Rushmore).

yellow = total length (µm)

red = head diameter (µm)

blue = tail length (µm)

Table 9 Summary of correlation between smoking habits and DNA damage among exposed workers.

	Exposed workers (n=32)		P value
Parameters	Smoker (n=10)	Non-smoker (n=22)	
Mean tail length (μm) (mean±S.D.)	5.97±6.65	5.30±4.99	0.952*
Mean tail moment (μm) (mean±S.D.)	2.03±3.24	1.77±2.45	0.952*
% of cell with aberration	2 %	1.82 %	0.483*

*Mann-Whitney U-test

Table 10 DNA damage of non-smoker in the petrol pump workers and control subjects.

	Non-smoker (n=51)		P value
Parameters	in Control group (n=29)	in Exposed group (n=22)	
Mean tail length (μm) (mean±S.D.)	1.60±1.04	5.30±4.99	0.000*
Mean tail moment (μm) (mean±S.D.)	0.31±0.27	1.77±2.45	0.000*
% of cell with aberration	1.03 %	1.82 %	0.048*

*Statistically significant (Mann-Whitney U-test).

Table 11 Summary of correlation between duration of exposure and DNA damage among exposed workers.

	Duration of exposure		P value
Parameters	3 months-1 year	1 year-5 years	
Number of subjects (n=32)	24	8	
Mean tail length (μm) (mean±S.D.)	5.84±5.82	4.54±4.40	0.380*
Mean tail moment (μm) (mean±S.D.)	1.96±2.81	1.51±2.35	0.380*
% of cell with aberration	1.83 %	2 %	0.623*

*Mann-Whitney U-test

Table 12 Comparison of DNA damage in the workers who had been exposed to 3-6 months and control subjects.

Parameters	Subjects		P value
	Controls	3-6 months of exposure	
Number of subjects	30	13	
Mean tail length (μm) (mean±S.D.)	1.57±1.03	5.21±6.12	0.001*
Mean tail moment (μm) (mean±S.D.)	0.31±0.27	1.71±2.90	0.004*
% of cell with aberration	1.07 %	1.38 %	0.488

*Statistically significant (Mann-Whitney U-test).

Table 13 Comparison of DNA damage in the workers who had been exposed for 6-12 months and control subjects.

Parameters	Subjects		P value
	Controls	6-12 months of exposure	
Number of subjects	30	11	
Mean tail length (μm) (mean±S.D.)	1.57±1.03	6.67±5.65	0.000*
Mean tail moment (μm) (mean±S.D.)	0.31±0.27	2.27±2.80	0.000*
% of cell with aberration	1.07 %	2.36 %	0.021*

*Statistically significant (Mann-Whitney U-test).

The present study found a statistically significant difference in both the structural chromosomal aberrations and the comet parameters between control subjects and petrol pump workers.