CHAPTER 5

RESULT

This chapter presents the results of the analyzed data set collected during the interview and the analyzed blood samples for polymorphism of GSTM1, GSTT1 and CYP1A1 of the case and control subjects.

5.1 Subjects recruited for the study

One hundred and two oral cancer patients were registered from the five selected centers in Jakarta during the study period. Out of these, 12 cases had to be excluded because they did not satisfy the criteria of oral squamous cell carcinoma (SCC) histopathologically, 9 cases were excluded due to incomplete data or due to completely damaged or lysed blood. Hence, 81 subjects with microscopically confirmed OSCC constituted the cases for this study.

Two hundred four eligible controls matched for age and sex as the cases were selected from the participating centers in the same time period (control patients were obtained within a maximum of 3 month after histopathological confirmation of the cases). When any controls have to be excluded due to incomplete data or complete damaged blood sample, they were replaced by other controls that fulfill the criteria. In addition, when a case becomes ineligible, the controls were also excluded. Subsequently 42 controls were excluded due to incomplete data in the questionnaire during samples collection, resulting in a total of 162 control patients.

5.2 Distribution of cases and controls by centers

The distribution of the cases and controls by study site is shown in Table 5.1. Majority of oral cancers (64.6%) and controls (48.8%) visited Ciptomangun kusumo Hospital (RSCM) which is located in central Jakarta. No oral cancer patients were from the Fatmawati Hospital (South Jakarta) during the study period.

Table 5.1 Distribution of case and control samples hospital-based

Centers	**Case (N=81)**		**Control (N=162)**	
	n	%	N	%
RSCM	52	64.6	79	48.8
RSGM Trisakti	21	25.6	75	46.3
RSGS	7	8.5	8	4.9
Ladogi TNI AL	1	1.2	0	0
RS Fatmawati	0	0	0	0
Total	81	100	162	100

5.3 Distribution of cases by anatomic sites

48 out of 81 cases of oral cancer were located on the tongue (59.3%, ICD-10 C01-C02). This was followed by the gingiva (19.8%, ICD-10 C03), and the buccal mucosa (11.1%, ICD-10 C06, C06.1-2, C06.8) and lip (4.9 %, ICD 10-C00). The occurrence of oral cancer at the other sites was less that 5 % (Figure 5.1). Fourty six (56.8%) of oral cancer cases were moderately differentiated Squamous Cell Carcinoma (SCC). Well differentiated SCC accounted for 17 cases (21%) with the remaining 18 (22.2%) being poorly differentiated SCC (Refer to Appendix J).

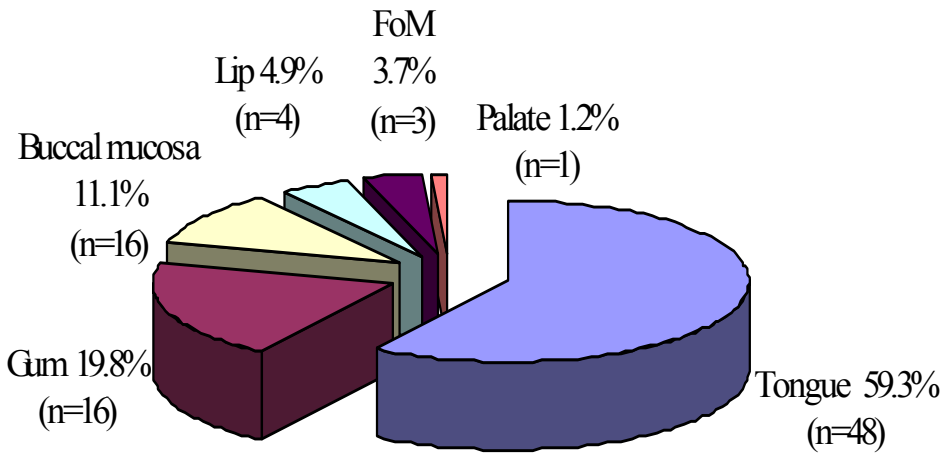

Figure 5.1 Sites of oral cancer (N=81)

5.4 Characteristics of the study population

The subject's characteristics are summarized in Table 5.2. The age range for oral cancer patients was 23-74 years (mean age 47.4 years ± 12.4), while that of the control group was 22-79 years (mean age 46.9 ± 11.8). The prevalence of oral cancer in the sample was higher in males than females with a ratio of 6:4.

The prevalence of oral cancer increases with increasing age-group with the highest prevalence recorded in the > 49 years age-group (43.2%). Ninety percent of cases were married, and the most affected ethnic group was Deutro Melayu (87.7 %) which comprised of those from Aceh, Sumatera (except Batak), Jawa, Bali, Sulawesi, Kalimantan, Betawi.

Table 5.2 Distribution of cases and controls by sociodemographic variables

	Control (N=162)		Cases (N=81)	
	N	%	N	%
Age group				
22-34	24	14.8	16	19.8
35-49	69	42.6	30	37.0
>49	69	42.6	35	43.2
Gender				
Male	100	61.7	50	61.7
Female	62	38.3	31	38.3
Marital status				
Married	134	82.7	73	90.1
Single	15	9.3	5	6.2
Divorced/Widow	13	8.02	3	3.07
Ethnic				
Deutro Melayu	134	82.72	71	87.7
Proto Melayu	10	6.17	7	8.6
Others	18	11.11	3	3.7

Similar trends were also observed among the control group with the exception that other ethnic groups account for a higher proportion among control group. However the difference between ethnic subgroups were not significant ($\chi 2$ = 4.0553 p value=0.132). The Chi^2 test of sociodemographic data is given in Appendix K.

5.5 Test reliability of scales

A set of new variables called Total Risk Factor (TRF), Total Daily Diet Pattern (TDDP), Total Food Preparation (TFP) and Total Food Frequency (TFF) was created by adding total scores for each subject under each construct in order to facilitate further analysis. The new variables were not tested for normality of distribution in the study population, since the nominal scale was entered for each scales. In order to ensure that the employed scales measure consistently what they are intended to measure, the Cronbach alpha coefficient was computed to check reliability. The value of normality and reliability test (Appendix L) is

illustrated in Table 5.3. The Cronbach alpha showed moderate values. As Peat (2001) deemed a scale to be moderately reliable, if Cronbach alpha ranges from 0-1 (0.4-0.6 is considered as moderate value).

Table 5.3 Normality and reliability test of scales

Variable/Scale	No. of Items	N	Mean	SD	Cronbach Alpha
TRF	3	243	4.31	1.31	0.409
TDDP	4	243	3.09	1.73	0.575
TFP	9	243	17.93	2.81	0.240
TFF	25	243	18.22	7.09	0.451

5.6 Distribution of cases and controls according to risk habits

The data from this study shows that 58 % of cases had risk habits as opposed to 51.9% of controls (Figure 5.2). Table 5.4 shows the distribution of habits among cases and controls. The most common habit (54.2 %) practiced among the cases was smoking, either as a single habit or in combination with alcohol and betel quid chewing. Alcohol drinking and betel quid chewing habits alone were only practiced by a small number of subjects (1.2% and 2.5%, respectively). There were no subjects who had both alcohol drinking and betel quid chewing habits. Combination of smoking and alcohol drinking habit was practiced in 12.3% of cases. The remaining combinations of habits (smoking and betel quid chewing or smoking, alcohol drinking and betel quid chewing) were practiced in less than 5% of cases.

Among controls who had habits, smoking was also the most common habit practiced (49.3%), either singly or in combination with alcohol drinking or betel

quid chewing habit. However there was no significant difference found in the frequency of smoking habit among cases and controls ($\chi 2$= 0.9963, p=0.318).

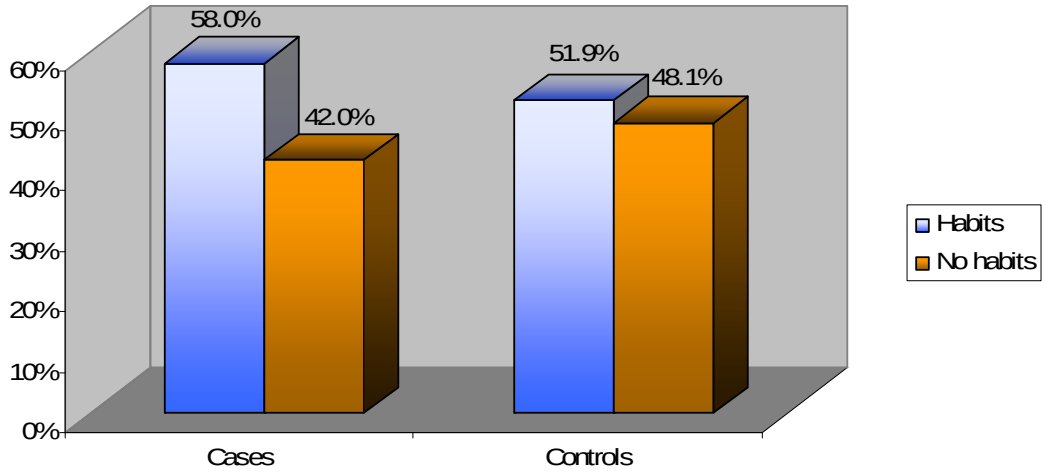

Figure 5.2. The percentage of cases and controls with and without habits

Table 5.4 Risk habits in study population

Risk Habits	Cases		Controls	
	n	%	n	%
No habit	34	42	79	48.8
Smoking	30	37	65	40.1
Alcohol	1	1.2	1	0.6
Betel quid chewing	2	2.5	2	1.2
Smoking & alcohol	10	12.3	14	8.6
Smoking & betel quid	3	3.7	0	0
Smoking, alcohol, betel quid	1	1.2	1	0.6
TOTAL	81	100	162	100

5.6.1 Smoking habits and risk of oral cancer

The smoking habit of the study population is shown in Figures 5.3 and 5.4. Most of the cases were smokers which accounted for 55.6%. On the other hand, those who never smoked constituted the majority among controls (51.2%). In term of the duration of smoking, the majority for both cases and controls had smoked more than 10 years. There were more cases who smoked a higher number of sticks per day as compared to controls. The majority of cases and control subjects used *kretek* cigarettes as compared to other types of cigarette.

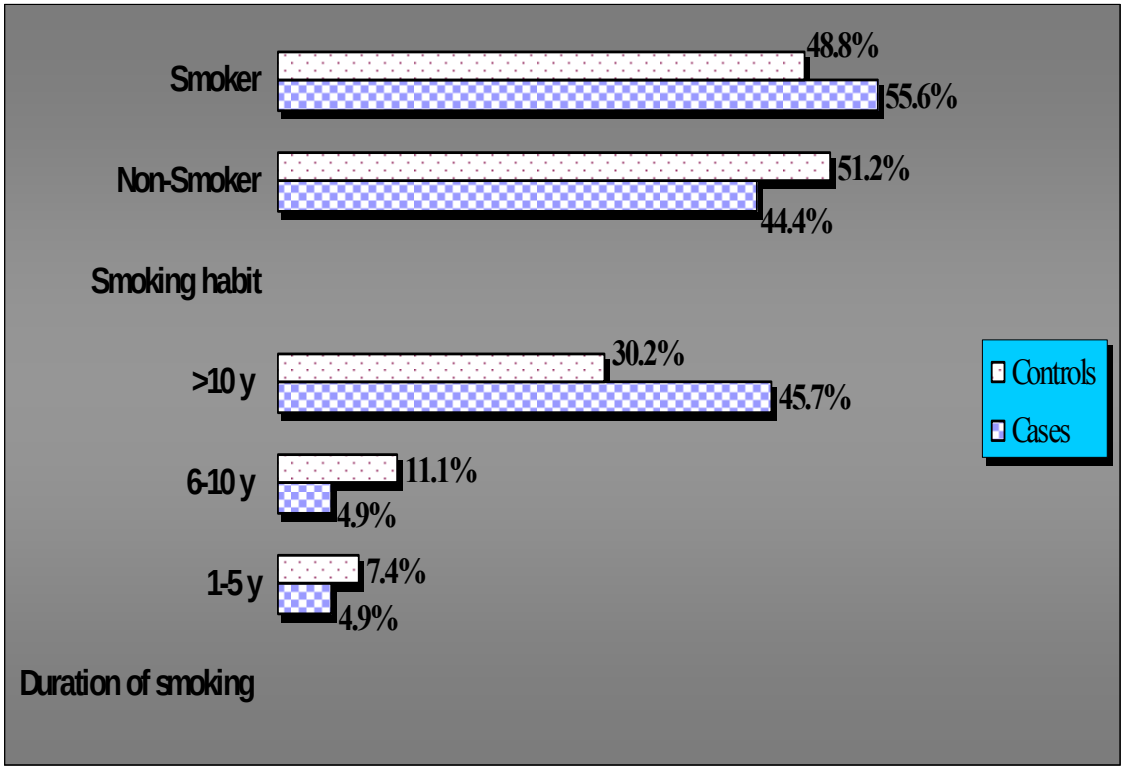

Figure 5.3 Distribution of cases and controls according to smoking status and duration of smoking

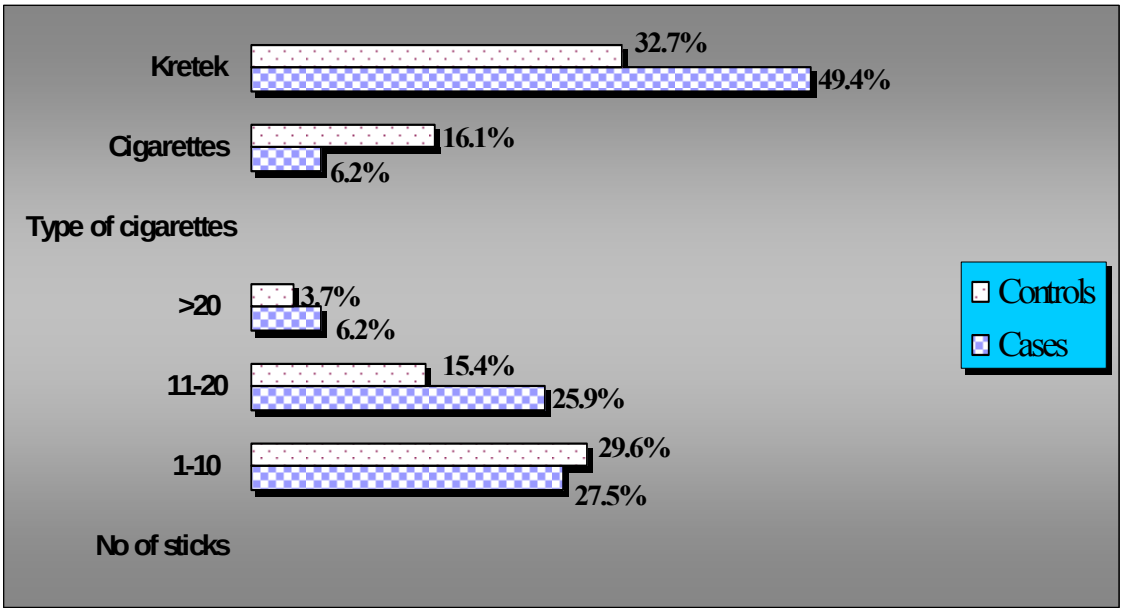

Figure 5.4. Distribution of cases and controls according to number of sticks smoked per day and types of tobacco used

The crude odd's ratios (OR) for oral cancer associated with smoking habit is shown in Table 5.5. The risk of developing oral cancer was higher among smokers (OR 1.58, 95% CI 0.81-3.09) compared to non smokers. However the difference was not statistically significant. This trend of increasing risk to oral cancer was also evident with increasing numbers of sticks smoked per day as well as with increasing duration of smoking. Again, the differences were not significant, except for those who smoked for more than 10 years. In terms of types of tobacco, the risk of oral cancer among *kretek* smoker doubled the risk of oral cancer compared to cigarettes (OR 1.91, 95% CI 0.98-3.95, p= 0.057). The Brickman Index showed that moderate and heavy smokers displayed significantly greater risk of oral cancer OR 2.31 (95% CI 1.24-10.78), OR 4.38, (95%CI 1.09-17.63), respectively. The statistical analysis for crude OR, is attached in Appendix M.

The adjusted odds ratios are shown in Table 5.6 after adjusting for the effects of the following confounders: marital status, alcohol drinking, betel quid chewing habit, CYP1A1, and daily diet pattern.

Table 5.5 Smoking habit and risk of oral cancer

Smoking habit	Disease status				Crude OR	95%CI	P
	Control (N=162)		Cases (N= 81)				
	n	%	n	%			
Smoking status							
Non smoker	83	51.2	36	44.4	1	Reference	-
Smoker	79	48.8	45	55.6	1.58	0.81-3.09	0.181
Number of sticks per day							
None	83	51.2	36	44.4	1	Reference	-
1-10 sticks	48	29.6	19	27.5	1.16	0.54-2.48	0.711
11-20 sticks	25	15.4	21	25.9	2.22	1.00-4.93	0.051
>20 sticks	6	3.7	5	6.2	2.35	0.60-9.20	0.221
Duration of smoking							
None	83	51.2	36	44.4	1	Reference	-
1-5 years	12	7.4	4	4.9	0.77	0.23-2.54	0.666
6-10 years	18	11.1	4	4.9	0.68	0.19-2.41	0.548
>10 years	49	30.2	37	45.7	2.25	1.06-4.78	0.034
Types of tobacco							
None	83	51.2	36	44.4	1	Reference	-
Cigarettes	26	16.1	5	6.17	0.52	0.17-1.62	0.260
Kretek	53	32.7	40	49.4	1.91	0.98-3.95	0.057
Brickman Index							
None	83	51.2	36	44.4	1	Reference	-
1-299 (Light)	61	37.6	25	30.9	1.17	0.57-2.42	0.673
300-599 (Moderate)	14	8.6	14	17.3	2.31	1.24-10.78	0.019
>599 (Heavy)	4	2.5	6	7.4	4.38	1.09-17.63	0.038

Almost all smoking habits (number stick per day, duration of smoking, and type of tobacco and pack-years of exposure of tobacco) displayed higher risks. Those who smoked 11-20 sticks of cigarette per day displayed about three times increase in risk of developing oral cancer. Similarly, those who smoked for more than 10 years increased their risk almost three-times. In terms of the types of tobacco, *kretek* also showed statistically significant risk after having adjusted for the variables mentioned (OR 2.57, 95%CI 1.20-5.48). Significantly, an increased in risk was also observed among moderate and heavy smoker where the adjusted

OR was about twice the crude OR. Please refer to Appendix N for statistical analysis of adjusted OR.

Table 5.6 Adjusted OR of smoking habits and risk for oral cancer

	Crude OR	95%CI	P	Adjusted OR	95%CI	P
Smoking status*						
Non smoker	1	Reference	-	1.00	Reference	
Smoker	1.58	0.81-3.09	0.181	2.09	1.01-4.32	0.049
Numbers of sticks*						
None	1	Reference	-	1.00	Reference	-
1-10 sticks	1.16	0.54-2.48	0.711	1.58	0.70-3.58	0.275
11-20 sticks	2.22	1.00-4.93	0.051	2.78	1.16-6.65	0.022
21-50 sticks	2.35	0.60-9.20	0.221	3.10	0.74-13.02	0.123
Duration of smoking*						
None	1	Reference	-	1.00	Reference	-
1-5 years	0.77	0.23-2.54	0.666	0.78	0.19-3.21	0.735
6-10 years	0.68	0.19-2.41	0.548	0.77	0.20-3.00	0.712
10-55 years	2.25	1.06-4.78	0.034	2.98	1.30-6.83	0.010
Types of tobacco*						
None	1	Reference	-	1.00	Reference	-
Cigarettes	0.52	0.17-1.62	0.260	0.69	0.21-2.25	0.543
Kretek	1.91	0.98-3.95	0.057	2.57	1.20-5.48	0.015
Brickman Index*						
None	1	Reference	-	1.00	Reference	-
1-299 (Light)	1.17	0.57-2.42	0.673	1.45	0.65-3.22	0.361
300-599 (Moderate)	2.31	1.24-10.78	0.019	4.47	1.42-14.11	0.011
600-1750 (Heavy)	4.38	1.09-17.63	0.038	4.80	1.10-20.95	0.037
* Adjusted for betel quid, diet pattern, and marital status.						

5.6.2 Alcohol habits and risk of oral cancer

Alcohol drinking habit was reported in less than 10% of cases and controls (8.6% and 4.3%, respectively). The analysis of risk of developing oral cancer due to alcohol consumption in case and control groups is shown in Table 5.7. Whilst current and former drinkers had an increased risk of oral cancer with OR 2.58 (95%CI 0.79-8.57) and OR 1.11 (95%CI 0.29-4.25) respectively compared to non drinkers, their differences were not statistically significant. The amount and duration of alcohol drinking also did not increase the risk of cancer.

Similarly, the types of alcohol consumed (beer, wine and whisky) did not increase the risk with the exception of wine where the risk increased by 11-times (OR 11.71, 95%CI 1.39-98.77), however, there were only 1 control and 6 cases thus rendering the analysis meaningless. As the univariate model for alcohol drinking habit showed that most of the parameters did not contribute to the risk of oral cancer (p>0.250), it has been excluded from the multivariate model and further analysis. The statistical analysis for crude OR is shown in Appendix O.

Table 5.7. Alcohol drinking habit and risk of oral cancer

Alcohol drinking habit	Disease status				Crude OR	95%CI	P
	Control (N=162)		Cases (N= 81)				
	n	%	n	%			
Alcohol drinking status							
Non drinker	147	90.7	70	86.4	1.00	Reference	
Drinker	15	9.3	11	13.6	1.77	0.69-4.51	0.231
Numbers of bottle per week							
None	149	92.0	70	86.4	1.00	Reference	
1-4 bottles/week	12	7.41	10	12.4	2.30	0.78-6.76	0.130
>5 bottles/week	1	0.62	1	1.23	2	0.13-31.97	0.624
Duration of alcohol drinking							
None	147	90.7	70	86.4	1.00	Reference	
1-5 years	10	6.2	5	6.2	1.14	0.36-3.57	0.828
6-10 years	2	1.2	1	1.2	1.04	0.09-11.92	0.972
11-55 years	3	1.9	5	6.2	8.11	0.90-72.81	0.062
Type of alcohol							
None	147	90.7	70	86.4	1.00	Reference	
Beer/Local/traditional	11	6.8	4	4.9	0.79	0.20-3.21	0.744
Whisky	3	1.9	1	1.2	1.09	0.10-11.50	0.941
Wine	1	0.6	6	7.4	11.71	1.39-98.77	0.024

5.6.3. Betel quid chewing habits and risk of oral cancer

The calculation of the risk of developing oral cancer associated with betel quid chewing habit is shown in Table 5.8. Betel quid chewing habit was practiced by a very small number of cases (7.4%) and controls (1.8%). Most subjects who chewed betel quid preferred a quid combination of betel leaf, tobacco, areca nut,

and lime. Betel quid chewing, number of quid per day and quid combination of betel leaf, tobacco, areca nut, and lime were associated significantly ($p<0.05$) with increasing risk of oral cancer (OR 4.19, 95% CI 1.05-16.82; OR 5.32, 95% CI 1.03-27.52, OR 4.19, 95% CI 1.05-16.82 respectively). The adjusted risk of oral cancer for those who was a quid chewer, who chewed a combination of quid and who consumed 1-10 quid per day showed slightly increased risk after allowing for confounders (Table 5.9). However, the duration of chewing betel quid for more than 1 year did not show increased risk in this study (OR 3.51, 95% CI 0.89-14.75), even after allowing for the confounding factors. The statistical analysis is attached in Appendix P.

Table 5.8 Betel quid chewing habit and risk of oral cancer

Betel quid chewing habit	Disease status				Crude OR	95%CI	P
	Control (N=162)		Cases (N= 81)				
	n	%	n	%			
Betel quid chewing status							
Non chewer	159	98.1	75	92.6	1.00	Reference	
Chewer	3	1.85	6	7.41	4.19	1.05-16.82	0.043
Numbers of betel quid/day							
None	160	98.8	76	93.8	1.00	Reference	
1-10 quid/day	2	1.2	5	6.2	5.32	1.03-27.52	0.046
Duration of betel quid chewing							
None	159	98.2	76	93.8	1.00	Reference	
> 1 years	3	1.2	5	6.2	3.51	0.89-14.75	0.086
Type of Quid							
None	159	98.2	75	92.6	1.00	Reference	
Betel leaf,tobacco,areca nut,lime	3	1.9	6	7.4	4.19	1.05-16.82	0.043

Table 5.9 Adjusted OR for betel quid chewing habit and risk of oral cancer

Betel quid chewing habit	Crude OR	95%CI	P	Adjusted OR	95%CI	P
Betel quid chewing status*						
Non chewer	1.00	Reference		1.00	Reference	
Chewer	4.19	1.05-16.82	0.043	4.59	1.11-18.91	0.035
Numbers of quid/day *						
None	1.00	Reference		1.00	Reference	
1-10 quid/day	5.32	1.03-27.52	0.046	5.97	1.08-33.04	0.041
Duration of betel quid chewing *						
None	1.00	Reference		1.00	Reference	
>1 years	3.51	0.89-14.75	0.086	3.99	0.90-17.58	0.068
Type of Quid*						
None	1.00	Reference		1.00	Reference	
Betel leaf, tobacco, areca nut, lime	4.19	1.05-16.82	0.043	4.74	1.13-19.89	0.033
*Adjusted for smoking, alcohol, and diet pattern						

5.6.4 Genetic polymorphisms and risk of oral cancer

The frequency distribution of genetic polymorphism of GSTM1, GSTT1, and CYP1A1 among cases and controls is displayed in Table 5.10. The detailed tabulation of the polymorphisms in GSTM1, GSTT1 or CYP1A1 of subjects is shown in Appendix J. The band showing polymorphism of GSTM1 and GSTT1 and CYP1A1 polymorphism using PCR and RFLP respectively are demonstrated in Figures 5.5-5.7. In this study, GSTM1 and GSTT1 null was slightly overrepresented among cases (60.5% and 45.7% respectively) compared to controls (55.6% and 41.4% respectively), however no statistically significant differences were observed between cases and controls ($\chi 2$ test is attached in Appendix Q). In contrast, the distribution of CYP1A1 polymorphism was higher among controls compared to cases (52.5 % versus 42.4 %).

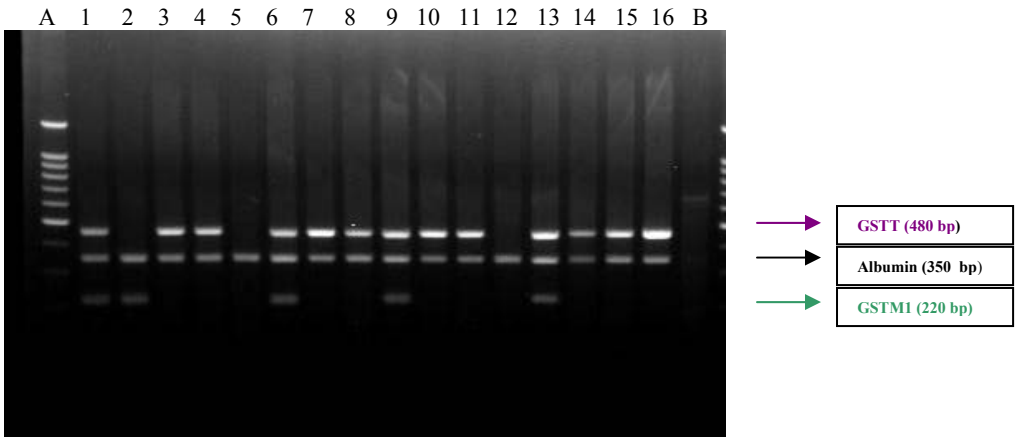

Figure 5.5 The GSTM1 deletion (null) was shown in lanes 3,4,5,7,8,10,11,12,14, 15,16 and GSTT1 null was shown in lane 2,5,12. GSTM1 wild type was shown in lanes 1,2,6,9,13 whereas GSTT1 wild type was shown in lanes 1,3,4,6,7,8,9,10, 11,13,14,15,16.

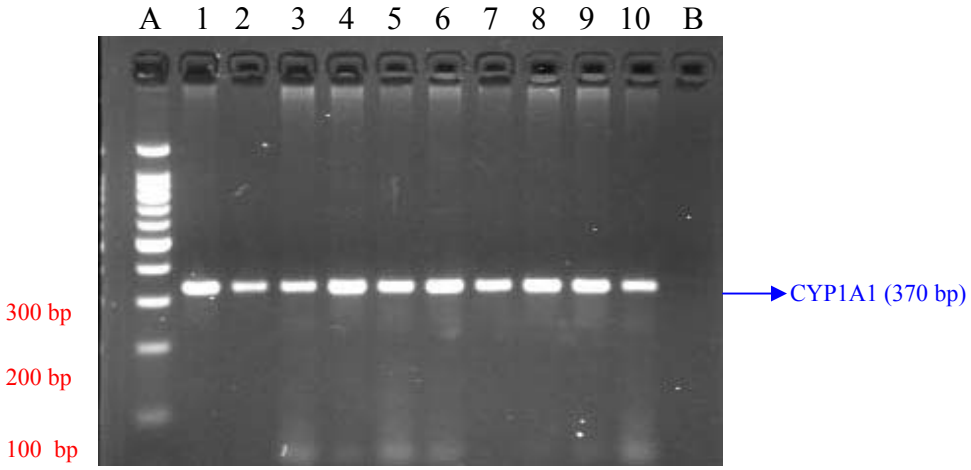

Figure 5.6 Lane A was molecular weight marker, lane B was negative control which showed absence of band. The CYP1A1 was evidence by the presence of band at 370 bp.

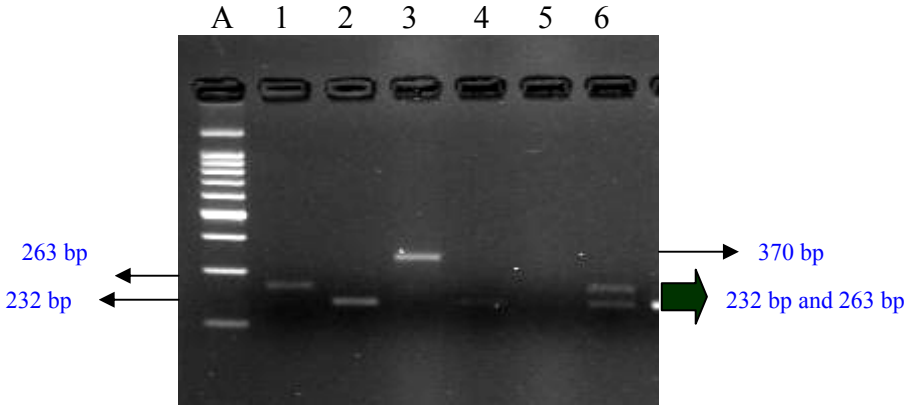

Figure 5.7 Other samples of RFLP for CYP1A1. Lane A contains DNA molecular weight markers. Lane 3 showed uncut CYP1A1 product (370 bp), lane 1 showed polymorphism bands at 263 bp (valine/valine). Lane 2 single bands at 232 bp for wild type allele of CYP1A1 and lane 6 showed polymorphism, consists of double bands at 263 bp and 232 bp (isoleusin/valine)

The ORs and 95% CI associated with GSTM1, GSTT1 or CYP1A1 polymorphism genotypes and oral cancer were calculated. The risk of null GSTM1 and GSTT1 genotypes was slightly higher compared to wild type genotypes (OR 1.19, 95% CI 0.70-2.02 and OR 1.19, 95% CI 0.72-2.05 respectively). However, it was not statistically significant ($p>0.05$). In term of the risk of combination of phase 1 (GSTM1 and GSTT1) and phase 2 (CYP1A1) genes; either two or three genes, the risk of oral cancer tend to increase, however it was not statistically significant.

In regard to smoking habit, single polymorphism of GSTM1 and GSTT1 or CYP1A1 had no influence on the occurrence of oral cancer among smokers (Table 5.11) or non smokers (Table 5.12). However, the combination of two or three genes polymorphism among non smokers tends to increase the risk but remained statistically insignificant. The statistical analysis is given in Appendix Q.

Genetic polymorphism of GSTM1, GSTT1 and CYP1A1 distribution among quid chewer was calculated and displayed in Table 5.13. Due to the small number of betel quid chewer in this population, the risk estimation could not be performed (Appendix Q). Additionally, Chi square test (Table 5.13) showed that there was no association between genetic polymorphism of the above genes (either single or combination polymorphism) among cases and controls who chewed betel quid and oral cancer ($p>0.05$).

Table 5.10 Genetic polymorphism and risk of oral cancer

Genetic polymorphism	Disease status				Crude OR	95% CI	P
	Control (N=162)		Case (N=81)				
	n	%	n	%			
GSTM1							
Expressed	72	44.4	32	39.5	1.00	Reference	-
Null	90	55.6	49	60.5	1.19	0.70-2.02	0.527
GSTT1							
Expressed	95	58.6	44	54.3	1.00	Reference	-
Null	67	41.4	37	45.7	1.19	0.72-2.05	0.463
CYP1A1							
Wild type	77	47.5	45	55.6	1.00	Reference	-
Polymorphism	85	52.5	36	44.4	0.70	0.39-1.25	0.226
Combination 2 genes							
GSTM/GSTT both wild type	41	25.3	17	21.0	1.00		
GSTM/GSTT either1 expressed	85	52.5	42	51.9	1.19	0.59-2.40	0.630
GSTM/GSTT both null	36	22.2	22	27.2	1.14	0.68-3.00	0.342
Combination 3 genes							
GTSM/GSTT/ CYP1A1 wild type	21	13.0	7	8.6	1.00		
GSTM/GSTT either one null and CYP1A1 polymorphism	65	40.1	34	42.0	1.74	0.63-4.84	0.287
GSTM/GSTT null and CYP1A1 wild type	51	31.5	32	39.5	2.06	0.76-5.60	0.158
All 3 genes polymorphism	25	15.4	8	9.9	1.05	0.34-3.28	0.934

Table 5.11 Distribution and risk of genetic polymorphism among smokers

Genetic polymorphism	Disease status				Crude OR	95% CI	P
	Control (N=79)		Case (N=45)				
	n	%	n	%			
GSTM1							
Wild type	38	48.1	21	46.7	1.00	Reference	
Null	41	51.9	24	53.3	1.09	0.50-2.40	0.839
GSTT1							
Wild type	42	53.2	22	48.9	1.00	Reference	
Null	37	46.8	23	51.1	0.97	0.43-2.19	0.945
CYP1A1							
Wild type	39	49.4	26	57.8	1.00	Reference	
Polymorphism	40	50.6	19	42.2	0.53	0.19-1.52	0.239
Combination 2 genes							
GSTM/GSTT both wild type	18	22.8	9	20	1.00	Reference	
GSTM/GSTT either one null	44	55.7	25	55.6	0.85	0.29-2.44	0.761
GSTM/GSTT both null	17	21.5	11	24.4	1.06	0.32-3.49	0.922
Combination 3 genes							
GTSM/GSTT/ CYP1A1 wild type	10	12.7	5	11.1	1.00	Reference	
GSTM/GSTT either one null and CYP1A1 polymorphism	31	39.2	17	37.8	0.52	0.11-2.56	0.420
GSTM/GSTT null and CYP1A1 wild type	27	34.2	20	44.4	0.68	0.14-3.33	0.639
All 3 genes polymorphism	11	13.9	3	6.7	0.37	0.05-2.62	0.314

Table 5.12 Distribution and risk of genetic polymorphism among Non-smokers

Genes	Disease status				Crude OR	95% CI	P
	Control (N=83)		Case (N=36)				
	n	%	n	%			
GSTM1							
Wild type	34	41.0	11	30.6	1.00	Reference	
Null	49	59.0	25	69.4	0.94	0.37-2.42	0.904
GSTT1							
Wild type	53	63.9	22	61.1	1.00	Reference	
Null	30	36.1	14	38.9	1.04	0.42-2.59	0.938
CYP1A1							
Wild type	38	45.8	19	52.8	1.00	Reference	
Polymorphism	45	54.2	17	47.2	1.11	0.41-3.04	0.833
Combination 2							
GSTM/GSTT both wild type	23	27.7	8	22.2	1.00		
GSTM/GSTT either1 null	41	49.4	17	47.2	0.70	0.21-2.40	0.573
GSTM/GSTT both null	19	22.9	11	30.6	0.92	0.29-2.95	0.884
Combination 3							
GTSM/GSTT/ CYP1A1 wild type	11	13.3	2	5.6	1.00		
GSTM/GSTT either one null and CYP1A1 polymorphism	34	41.0	17	47.2	5.17	0.45-59.38	0.187
GSTM/GSTT null and CYP1A1 wild type	24	29.0	12	33.3	4.96	0.44-55.37	0.194
All genes polymorphism	14	16.87	5	13.9	2.81	0.25-23.49	0.446

Table 5.13 Distribution of genetic polymorphism among Quid chewers

Genes	Control (N=162)		Case (N=81)		χ 2	P value
	n	%	n	%		
GSTM					3.600	0.058
Wild type	0	0.0	4	66.7		
Polymorphism	3	100.0	2	33.3		
GSTT					0.321	0.571
Wild type	1	33.3	1	16.7		
Polymorphism	2	66.7	5	83.3		
CYP1A1					0.000	1.000
Wild type	2	66.7	4	66.7		
Polymorphism	1	33.3	6	100.0		
Combination 2					1.1250	0.570
GSTM/GSTT both wild type	0	0.0	1	16.7		
GSTM/GSTT either1 null	1	33.3	3	50.0		
GSTM/GSTT both null	2	66.7	2	33.3		
Combination 3						
GTSM/GSTT/ CYP1A1 wild type	0	0.0	1	16.7	0.7500	0.861
GSTM/GSTT either one null and CYP1A1 polymorphism	1	33.3	2	33.3		
GSTM/GSTT null and CYP1A1 wild type	1	33.3	2	33.3		
All genes polymorphism	1	33.3	1	16.7		

Table 5.14 displays the distribution and risk of genetic polymorphism among cases and controls who were non betel quid chewer. In this population, the increased risk for oral cancer was higher in those who had combined polymorphism of GSTM1, GSTT1 with wild type if CYP1A1 genes, however it was statistically not significant.

Table 5.14 Distribution of genetic polymorphism among Non-Quid chewers

Genes	Disease status				Crude OR	95% CI	P
	Control (N=83)		Case (N=36)				
	n	%	n	%			
GSTM1							
Wild type	72	45.3	28	37.2	1.00	Reference	
Null	87	54.7	47	62.7	1.36	0.78-2.34	0.276
GSTT1							
Wild type	94	59.1	43	57.3	1.00	Reference	
Null	65	40.9	32	42.7	1.16	0.67-2.01	0.594
CYP1A1							
Wild type	75	47.2	41	54.7	1.00	Reference	
Polymorphism	84	52.8	34	45.3	0.73	0.40-1.33	0.303
Combination 2							
GSTM/GSTT both wild type	41	25.8	16	21.3	1.00	Reference	
GSTM/GSTT either1 null	84	52.8	39	52.0	1.14	0.55-2.37	0.719
GSTM/GSTT both null	34	21.4	20	26.7	1.53	0.72-3.25	0.271
Combination 3							
GTSM/GSTT/ CYP1A1 wild type	21	13.2	6	8.0	1.00	Reference	
GSTM/GSTT either one null and CYP1A1 polymorphism	64	40.3	32	42.7	1.90	0.64-5.63	0.248
GSTM/GSTT null and CYP1A1 wild type	50	31.5	30	40.0	2.39	0.83-6.85	0.106
All genes polymorphism	24	15.1	7	9.3	1.16	0.32-3.83	0.805

5.6.5 Dietary habits and risk of oral cancer

Information on dietary pattern for this study was obtained using the Food Frequency Questionnaire (FFQ). Dietary items were divided into 3 groups (dietary habit, food preparation and food frequency questionnaire) according to the respondent's frequency of intake. These groups were further divided into three subgroups: (1) low, if the dietary intake indicated is never, seldom or 2-3 times per month, (2) medium, if the intake is 1-2 times per week and (3) high, if the intake is 1-3 times per day.

The general overview of dietary habits of the subjects is illustrated in Table 5.15. There was no difference between cases and controls for non-vegetarians (96.3% and 91.9%, respectively, $p>0.05$). Most of the cases consumed high frequency of non home cooked and fast food compared to controls ($p=0.000$). In contrast, there was no difference between cases and controls in frequency of consumption of frozen food and the reuse of oil ($p> 0.05$) for cooking (Appendix R).

In terms of food preparation (steamed, fried, barbequed, roasted and other servings), no significant difference was observed between cases and controls ($p>0.05$) (Table 5.16), except for the consumption of chicken in fried form which offered a protective effect (OR 0.43, 95% CI 0.20-0.90). On the other hand, the risk increased two-fold when vegetables were consumed fried instead of steamed (OR 2.04, 95% CI 1.11-3.74). The statistical analysis is attached in Appendix S.

Table 5.15 Frequency distribution of dietary habits in the study population

	Control (N=162)		Case (N=81)		$\chi 2$	P value
	n	%	n	%		
Daily food pattern					1.639	0.200
Ovo/Ovolacto vegetarian	13	8.0	3	3.7		
Non vegetarian	149	91.9	78	96.3		
Frequency of non home cooked					37.050	0.000
Low	45	55.6	29	17.9		
Medium	10	12.4	50	30.9		
High	26	32.1	83	51.2		
Frequency of fast food					17.962	0.000
Low	53	65.4	61	37.7		
Medium	9	11.1	46	28.4		
High	19	23.5	55	34.0		
Frequency of frozen food					2.938	0.230
Low	131	81.4	72	88.9		
Medium	27	16.3	7	8.6		
High	3	1.9	2	2.5		
Frequency of reused oil					1.7465	0.418
1-2 times	124	76.5	63	77.8		
3-4 times	37	22.8	16	19.8		
>5 times	1	0.6	2	2.5		

5.6.5.1 Factor analysis of dietary pattern

The observed Kaiser-Meyer-Olkin (KMO), a measurement of sampling adequacy was 0.671 (more than 0.600, Pallant, 2005) and Bartlett's test of sphericity was less than 0.05. This meant that the sample was adequate for factor analysis. Four components were identified through factor analysis based on the Kaiser Criterion (eigenvalue more than 1) and Scree plot (Figure 5.8). These four components accounted for 55% of the variability within the samples (Appendix S). Table 5.17 shows the loading factors obtained after orthogonal varimax rotation. Factor loadings obtained for each dietary variable in each factor of more than

0.40 (for sample size 200-249) have been highlighted and are considered as having significantly contributed to the factor.

Table 5.16 Method of food preparation and risk for oral cancer in the study population

Food preparation	Control (N=162)		Case (N=81)		OR	CI	P
	n	%	n	%			
Chicken							
Steam	21	13.0	19	23.5	1.00	Reference	-
Fried	131	80.9	54	66.7	0.43	0.20-0.90	0.026
Barbeque	4	2.5	3	3.7	0.73	0.14-3.90	0.715
Roast	3	1.9	1	1.2	0.33	0.03-3.69	0.371
Others	3	1.9	4	4.9	1.34	0.26-6.90	0.723
Beef							
Steam	117	72.2	49	60.5	1.00	Reference	-
Fried	31	19.1	18	22.2	1.56	0.73-3.32	0.247
Barbeque	2	1.2	1	1.2	1.00	0.09-11.03	1.000
Roast	2	1.2	2	2.5	2.13	0.30-15.19	0.452
Others	10	6.2	9	11.1	2.27	0.86-5.99	0.098
Fish							
Steam	11	6.8	10	12.3	1.00	Reference	-
Fried	136	84.0	61	75.3	0.47	0.16-1.14	0.095
Barbeque	8	4.9	3	3.7	0.38	0.08-1.79	0.222
Roast	3	1.9	2	2.5	0.75	0.11-5.08	0.766
Others	4	2.5	5	6.2	1.55	0.31-7.76	0.592
Egg							
Steam	34	21.0	17	21.0	1.00	Reference	-
Fried	127	78.4	63	77.8	0.98	0.49-1.97	0.952
Others	1	0.6	1	1.2	1.96	0.11-34.15	0.645
Vegetables							
Steam	113	69.8	46	56.8	1.00	Reference	-
Fried	42	25.9	32	39.5	2.04	1.11-3.74	0.021
Others	7	4.3	3	3.7	0	0.25-3.76	0.959

The first component (factor), which accounted for 27% of the total variance, is labelled as “preferred”. Fast food, fermented food, canned food, snacks high in fat and sugar, cooked and raw vegetables, and seafood fall into this component. The second factor explained 11% of the total variance. This factor was loaded by the intake of dairy product, red meat, white meat and fruit; it was labelled “combination”. The third

factor accounted for approximately 9% of the total variance. In this component high loading factor was observed in the processed food and monosodium glutamate (MSG). This factor was labelled “chemical related”. The fourth principle component approximately accounted for 8% of the total variance was loaded by two food groups, drinks and grain. This factor has been labelled “traditional”.

The highest communality was shown in “Fast food” (0.818) which indicates that this variable has much in common with other variables taken as a group and loaded in component or factor 1 as labelled “preferred”.

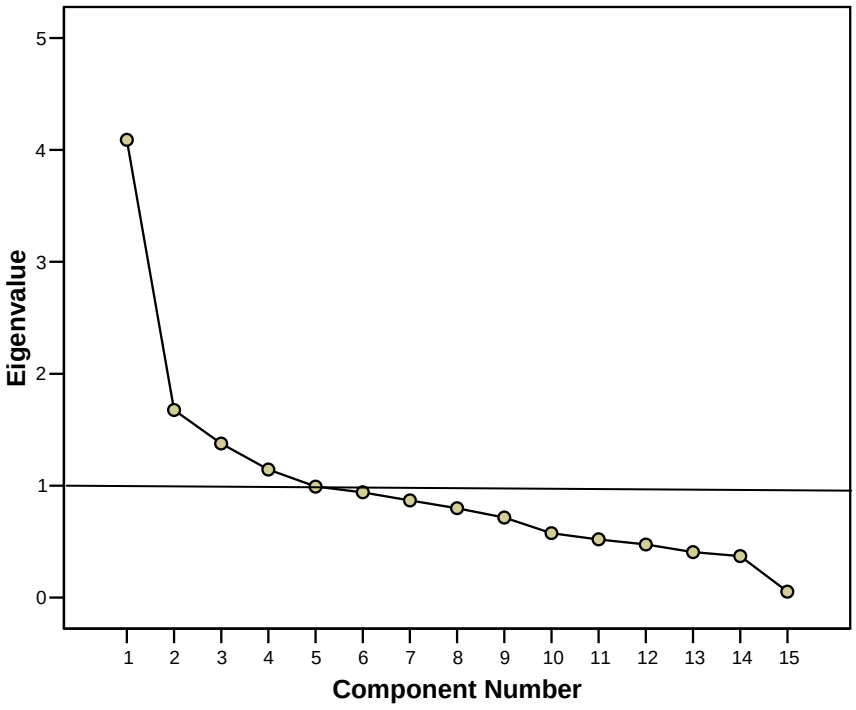

Figure 5.8 Scree plot showing *eigenvalues* for 15 components, in the factor extraction of data obtained from food frequency questionnaire

Table 5.17. Food group factor loadings for the four dietary patterns identified in the study population

Food group	Dietary pattern				Communality
	Preferred food	Combination	Chemical related	Traditional	
Fast food	**0.749**	-0.018	0.463	0.206	**0.818**
Cooked Vegetables	**0.737**	0.155	-0.055	-0.144	0.591
Raw vegetables	**0.709**	0.015	0.152	-0.039	0.528
Fermented food	**0.690**	-0.040	0.450	0.013	0.681
Seafood	**0.621**	0.250	-0.205	0.028	0.491
Canned food	**0.572**	-0.082	-0.260	0.261	0.469
Snack high fat and sugar	**0.483**	0.307	0.402	0.285	0.570
Red meat	0.098	**0.730**	0.212	-0.065	0.591
Dairy product	-0.084	**0.722**	-0.149	0.173	0.580
Fruits	0.522	**0.584**	-0.175	0.027	0.645
White meat	0.115	**0.545**	0.395	-0.204	0.508
Processed food	0.015	0.082	**0.588**	-0.040	0.354
MSG	-0.118	-0.156	**0.467**	0.451	0.460
Drinks	-0.027	0.028	-0.095	**0.723**	0.534
Grain	0.418	0.077	0.127	**0.518**	0.465
Eigenvalue	*4.090*	*1.676*	*1.376*	*1.144*	
% explained variance	*27.264*	*11.174*	*9.175*	*7.624*	
% cumulative variance	*27.264*	*38.438*	*47.613*	*55.237*	

The $\chi 2$ test for the upper limit of intake of four components (called preferred food, combination, chemical related and traditional) retained from factors analysis was performed and showed that all the components were statistically significant contribution to development of oral cancer, $p<0.05$ (Appendix T).

Table 5.18 illustrates the upper limit of intake tertile of each food group retained from factor analysis and corresponding univariate analysis and adjusted Odds ratio were done after allowing for confounding factors (Appendix T).

Consumption in the highest tertile of the "preferred" pattern was shown to increase the risk of oral cancer by two-times (adjusted OR 2.17, 95%CI 1.05-4.50, $\chi 2$ trend 5.446, $p<0.05$).

The intake of the highest tertile of "chemical related" pattern was found to be associated with higher risk of about 3-fold after adjusting for controls (adjusted

OR 2.85, 95%CI 1.34-6.05, $\chi 2$ trend 5.640, $p<0.05$).

On the other hand, the highest tertile of "combination" pattern displayed protective effects (64%) in relation to oral cancer before or after adjusting for the variables mentioned earlier (adjusted OR 0.46, 95% CI 0.23-0.91, $\chi 2$ trend 7.335, $p<0.01$).

Consumption of highest tertile of "traditional" pattern showed an increased of risk by two-fold (OR 2.10, 95%CI 1.02-4.30, $\chi 2$ trend 5.649, $p<0.05$) after allowing for ethnic, and dietary intake habit.

Table 5.18 Odds ratio and 95% CI for oral cancer, in approximate tertile for food groups defined by factor analysis

Factor	**Tertile of score**	**Control:Cases**	**Crude OR (95% CI)**	**Adjusted OR (95%CI)**	**$\chi 2$ p trend**
Preferred §	1st (5.1)	58:23	1.00	1.00	5.446*
	2nd (8.0)	58:23	1.04 (0.52-2.07)	1.05 (0.53-2.28)	
	3rd	58:46	1.89 (0.98-3.64)	2.17 (1.05-4.50)	
Combination†	1st (1.7)	42:39	1.00	1.00	7.335**
	2nd (3.0)	61:19	0.35 (0.17-0.70)	0.36 (0.18-0.75)	
	3rd	59:23	0.42 (0.22-0.81)	0.46 (0.23-0.91)	
Chemical related†	1st (2.0)	67:22	1.00	1.00	5.640*
	2nd (2.0)	54:24	1.43 (0.67-3.07)	1.48 (0.67-3.28)	
	3rd	41:35	2.56 (1.18-5.54)	2.85 (1.34-6.05)	
Traditional†	1st (4.0)	60:22	1.00	1.00	5.649*
	2nd (5.0)	57:23	1.15 (0.57-2.26)	1.22 (0.59-2.53)	
	3rd	45:36	2.09 (1.07-4.06)	2.10 (1.02-4.30)	

§ Adjusted for smoking status, smoking duration, type of tobacco, number stick of cigarettes, pack-years
† Adjusted for smoking, alcohol and betel quid
* $p<0.05$, ** $p<0.01$