RISK FACTORS AND GENETIC POLYMORPHISMS OF GSTM1, GSTT1 AND CYP1A1 IN DEVELOPMENT OF ORAL CANCER IN AN INDONESIAN POPULATION

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Introduction
Cancer is one of the foremost causes of death after infections and heart diseases in all societies and is the fatal leading disease in the world. Oral cancer is the sixth most common cancer worldwide and the most common in South East Asia. The geographic pattern and trends in incidence of oral cancer vary widely between countries and geographical areas of the world. This difference in distribution of oral cancer worldwide is influenced by their risk factors. The major risk factors of oral cancer which have already been established are tobacco use, alcohol drinking and betel quid chewing. Furthermore, epidemiology studies have also implicated other factors such as genetic susceptibility and the role of diet. Jakarta, the capital city of Indonesia is the most populous place in Indonesia. It is inhabited with more than 50% of Indonesia’s population. Jakarta city comprises of inhabitants of various ethnicity and practicing different habits. To date no studies have been undertaken to determine the risk factors including genetic polymorphism of metabolizing gene (GSTM1, GSTT1 and CYP1A1) in the development of oral cancer in the Jakarta population.

Objective/Aim
This study aims to identify and determine the risk factors including genetic polymorphisms in GSTM1, GSTT1 and CYP1A1 in the development of oral cancer in a Jakarta population, Indonesia.

Material and Method
This is a cross sectional study with case-control design involving hospital-based subjects matched for age and sex. Two hundred forty three subjects participated in this study from 5 selected hospitals which represent each district of Jakarta, with a 1:2 ratio of cases and
controls. This study consists of two parts. The first part consists of data collection on the subjects’ socio demographic characteristics, risk habits (smoking, alcohol consumption and betel quid chewing) and dietary pattern using a structured questionnaire. The second part consists of laboratory work on blood samples of cases and controls to assess the polymorphism of GSTM1, GSTT1 and CYP1A1 by means of PCR and Restriction Fragment Length Polymorphism (RFLP). The data was analysed using conditional logistic regression (STATA 8) and factor analysis (SPSS12) for dietary pattern.

**Results**

In terms of socio demographic characteristic, 42.3% of oral cancer cases were more than 49 years of age (mean 47.4 years ± 12.4), males being more frequently affected than females with a ratio of 6:4 and the number increases with increasing age-group. Ninety percent of cases were married, and the most affected ethnic group was Deutro Melayu (87.7%).

The most common habit practiced among the cases (54.2%) and controls (49.3%) was smoking, either as a single habit or in combination with alcohol and betel quid chewing. Smokers accounted for 55.6% of the cancer cases whilst there was an almost similar proportion of smoker and non smoker among the controls (48.8% cases vs 51.2% controls). Among cases and controls, the majority had smoked for more than 10 years. *Kretek* was the most preferred type of cigarettes for both cases and controls. Almost all smoking factors (number of sticks per day, duration of smoking, and type of tobacco and pack-years of exposure of tobacco) displayed higher risks of oral cancer by two to three times after allowing for confounding factors.

Alcohol drinking habit was practiced by less than 10% of case and control groups (8.6% and 4.3%, respectively). The crude OR showed that alcohol drinking habit did not
contribute to the risk of oral cancer (p>0.250). The exception was that those who consumed wine had 11 times higher risk of oral cancer.

Similarly, betel quid chewing habit was also practiced by a very small number of case (7.4%) and control subjects (1.8%). The findings showed that duration of chewing and number of quid per day did not significantly contribute to the risk of oral cancer. However, current betel quid chewer and quid combination of betel leaf, tobacco, areca nut, and lime were significantly associated (p<0.05) with increasing risk of oral cancer (OR 5.32, 95% CI 1.03-27.52; OR 4.19, 95% CI 1.05-16.82 respectively).

The genetic polymorphism of GSTM1, GSTT1 and CYP1A1 assay showed that there was no statistically significant difference observed between cases and controls. The polymorphism of GSTM1, GSTT1 and CYP1A1 either singly or in combination did not have association with risk of oral cancer even in the smoker, non smoker as well as among betel quid chewer and non chewer in this study.

Analysis of dietary pattern found four factors retained from factor analysis, referred to as preferred food, combination, chemical related and traditional that were associated with oral cancer after adjusting for smoking, alcohol and betel quid chewing. The preferred food consisted of fast food, fermented food, canned food, snacks high in fat and sugar, cooked and raw vegetables, and seafood showed increased risk of oral cancer by two times (OR 2.17, 95% CI 1.05-4.50, $\chi^2$ trend 5.446, p<0.05). The “chemical related” pattern (which is consisted of MSG and processed food) also showed an increase risk (OR 2.85, 95% CI 1.34-6.05). The “combination” pattern (consisted of meat, dairy product and fruit) displayed protective effects in relation to oral cancer (adjusted OR 0.46, 95% CI 0.23-0.91, $\chi^2$ trend 7.335, p<0.01). Finally, “traditional” pattern (consist of drinks and carbohydrate)
showed an increased of risk by two-fold (OR 2.04, 95%CI 1.01-4.41, \( \chi^2 \) trend 5.649, \( p<0.05 \)). The fast food was the most common food type consumed in this study (Communality : 0.818)

**Conclusion**

Smoking and betel quid chewing were found to be risk factors for oral cancer in the Jakarta population. In contrast genetic polymorphism of GSTM1, GSTT1 and CYP1A1 did not contribute to the development of oral cancer in this study. The dietary pattern referred to as “preferred food”, “chemical related” and “traditional” were found to be risk factors of oral cancer whereas the “combination” pattern had protective effect of oral cancer in this study.

**Recommendations**

Future research is recommended to include a bigger sample size and greater cross section that constitute the Indonesian population and using community controls. Investigation involving nutritional content of food consumed is also advocated.
# LIST OF CONTENT

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVER PAGE</td>
</tr>
<tr>
<td>ABSTRACT</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
</tr>
<tr>
<td>CHAPTER 1 INTRODUCTION</td>
</tr>
<tr>
<td>CHAPTER 2 PURPOSE OF STUDY</td>
</tr>
<tr>
<td>CHAPTER 3 LITERATURE REVIEW</td>
</tr>
</tbody>
</table>
  - 3.1 Terminology and classification of oral cancer | 9          |
  - 3.2 Epidemiology of oral cancer | 13         |
    - 3.2.1. Global epidemiology of cancer | 13         |
    - 3.2.2. Incidence of oral cancer in the world | 17         |
    - 3.2.3. Incidence of oral cancer in developed countries | 18         |
    - 3.2.4. Incidence of oral cancer in developing countries | 19         |
    - 3.2.5. Oral cancer mortality | 21         |
    - 3.2.6. Gender distribution of oral cancer | 22         |
    - 3.2.7. Age distribution of oral cancer | 23         |
    - 3.2.8. Ethnic distribution of oral cancer | 24         |
3.3 Clinical and Pathologic Characteristic of Oral Cancer and Precancer

3.3.1 Clinical presentation of oral cancer

3.3.1.1 White lesion

3.3.1.2 Red lesion

3.3.1.3 Ulcerated lesion

3.3.1.4 Exophytic lesion

3.3.2 Clinical and Pathologic Characteristic of Precancer (Potentially Malignant Lesions/Conditions)

3.3.2.1 Leukoplakia

3.3.2.2 Erythroplakia

3.3.2.3 Malignant potential of precancer and other lesions/conditions

3.3.3 Histopathology of oral cancer

3.3.4 Sites of cancer

3.3.4.1 Tongue cancer

3.3.4.2 Floor of the mouth

3.3.4.3 Buccal mucosa and lip

3.3.4.4 Gum and palate

3.4 Carcinogenesis and Risk Factors

3.4.1 Carcinogenesis

3.4.2 Risk Factors

3.4.2.1 Tobacco use as a risk factor

3.4.2.1.1 Carcinogenicity of tobacco

3.4.2.1.2 to oral tissue
carcinogenesis
CHAPTER 4 MATERIALS AND METHODS

4.1 Study design

4.2 Conduct of study

4.3 Recruitment of subjects
    4.3.1 Cases
    4.3.2 Controls
    4.3.3 Number of subjects in study population

4.4 Questionnaire survey
    4.4.1 Components in questionnaire survey
        4.4.1.1 Risk factor
            4.4.1.1.1 Smoking
            4.4.1.1.2 Alcohol consumption
            4.4.1.1.3 Quid chewing
            4.4.1.1.4 Dietary habit
                a. Daily food pattern
                b. Food preparation method
                c. Food frequency
        4.4.1.2 Validation of questionnaire
        4.4.1.3 Pre-test of questionnaire
        4.4.1.4 Conduct of questionnaire

4.5 Laboratory procedure for genetic polymorphism
    4.5.1 Tissue collection and storage
4.5.2. Blood collection and DNA isolation

4.5.2.1. Genotyping assay of GSTM1 and GSTT1 102

4.5.2.2. Genotyping assay of CYP1A1 106

4.6 Research ethics 109

4.7 Data analysis 109

CHAPTER 5 RESULTS

5.1 Subjects recruited for study 113

5.2 Distribution of cases and controls by centers 114

5.3 Distribution of cases by anatomic sites 114

5.4 Characteristic of study population 115

5.5 Test reliability test of scales 116

5.6 Distribution of cases and controls according to risk habit 117

5.6.1 Smoking habit and risk of oral cancer 119

5.6.2 Alcohol habits and risk of oral cancer 122

5.6.3 Betel quid chewing habit and risk of oral cancer 123

5.6.4 Genetic polymorphism and risk of oral cancer 125

5.6.5 Dietary habits and risk of oral cancer 133

5.6.5.1 Factor analysis of dietary pattern 134

CHAPTER 6 DISCUSSION

6.1 Limitation of study 139

6.2 Hypothesis 142

6.3 Socio demographic characteristic of Jakarta population 143

6.4 Habits in Jakarta population and risk of oral cancer 145

6.4.1 Smoking habit and risk of oral cancer 145
6.4.2 Alcohol drinking habit and risk of oral cancer 148
6.4.3 Betel quid chewing habit and risk of oral cancer 150
6.4.4 Genetic polymorphism and risk of oral cancer 153
6.4.5 Dietary habits and risk of oral cancer 158

CHAPTER 7 CONCLUSION AND RECOMMENDATIONS 166
7.1 Conclusion 166
7.1.1 Tobacco smoking 167
7.1.2 Alcohol drinking 167
7.1.3 Betel quid chewing 168
7.1.4 Genetic polymorphism of GSTM1, GSTT1 and CYP1A1 168
7.1.5 Dietary pattern 168
7.2 Recommendation 169

BIBLIOGRAPHY 171

APPENDICES 196
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Estimated Numbers of New Cancer Cases (Incidence) and Deaths (Mortality) in 2002. Data shown in thousands for developing and developed countries by cancer site and sex</td>
<td>16</td>
</tr>
<tr>
<td>3.2</td>
<td>Multistep carcinogenesis: initiation, promotion and progression</td>
<td>39</td>
</tr>
<tr>
<td>3.3</td>
<td>Indonesia Island</td>
<td>85</td>
</tr>
<tr>
<td>4.1</td>
<td>Flowchart of data collection</td>
<td>94</td>
</tr>
<tr>
<td>5.1</td>
<td>Sites of oral cancer</td>
<td>115</td>
</tr>
<tr>
<td>5.2</td>
<td>Distribution of cases and control with and without habit</td>
<td>118</td>
</tr>
<tr>
<td>5.3</td>
<td>Distribution of cases and controls according to smoking status and duration of smoking</td>
<td>119</td>
</tr>
<tr>
<td>5.4</td>
<td>Distribution of cases and controls according to number of stick smoked per day and types of tobacco used</td>
<td>120</td>
</tr>
<tr>
<td>5.5</td>
<td>A Multiplex PCR reaction of GTSM1 and GSTT11 polymorphism</td>
<td>126</td>
</tr>
<tr>
<td>5.6</td>
<td>CYP1A1 products (370 bp).</td>
<td>126</td>
</tr>
<tr>
<td>5.7</td>
<td>Restriction Fragment Length Polymorphism (RFLP) for CYP1A1 using Nco1 enzyme.</td>
<td>126</td>
</tr>
<tr>
<td>5.8</td>
<td>Scree plot showing eigenvalues for 15 components, in the factor extraction of data obtained from food frequency questionnaire</td>
<td>136</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Table 3.1: Incidence and Mortality by Sex and Cancer Site Worldwide in year 2002</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>Table 3.2: Some common forms of oral smokeless tobacco</td>
<td>60</td>
</tr>
<tr>
<td>3.</td>
<td>Table 5.1: Distribution of cases and controls by hospital location</td>
<td>114</td>
</tr>
<tr>
<td>4.</td>
<td>Table 5.2: Distribution of cases and controls by socio demographic variables</td>
<td>116</td>
</tr>
<tr>
<td>5.</td>
<td>Table 5.3: Normality and reliability test of scales</td>
<td>117</td>
</tr>
<tr>
<td>6.</td>
<td>Table 5.4: Risk habits in study population</td>
<td>118</td>
</tr>
<tr>
<td>7.</td>
<td>Table 5.5: Smoking characteristics and risk of oral cancer</td>
<td>121</td>
</tr>
<tr>
<td>8.</td>
<td>Table 5.6: Adjusted OR for smoking</td>
<td>122</td>
</tr>
<tr>
<td>9.</td>
<td>Table 5.7: Alcohol consumption status and risk of oral cancer</td>
<td>123</td>
</tr>
<tr>
<td>10.</td>
<td>Table 5.8: Betel quid chewing status and risk of oral cancer</td>
<td>124</td>
</tr>
<tr>
<td>11.</td>
<td>Table 5.9: Adjusted OR for betel quid chewing status and risk of oral cancer</td>
<td>125</td>
</tr>
<tr>
<td>12.</td>
<td>Table 5.10: Genetic polymorphism and risk of oral cancer</td>
<td>128</td>
</tr>
<tr>
<td>13.</td>
<td>Table 5.11: Distribution and risk of genetic polymorphism among smoker</td>
<td>129</td>
</tr>
<tr>
<td>14.</td>
<td>Table 5.12: Distribution and risk of genetic polymorphism among Non-smoker</td>
<td>130</td>
</tr>
<tr>
<td>15.</td>
<td>Table 5.13: Distribution of genetic polymorphism among quid chewer</td>
<td>131</td>
</tr>
<tr>
<td>16.</td>
<td>Table 5.14: Distribution of genetic polymorphism among non quid chewer</td>
<td>132</td>
</tr>
<tr>
<td>17.</td>
<td>Table 5.15: Frequency distribution of dietary habit in the study population</td>
<td>134</td>
</tr>
<tr>
<td>18.</td>
<td>Table 5.16: Method of food preparation and risk of oral cancer in the study population</td>
<td>135</td>
</tr>
<tr>
<td>19.</td>
<td>Table 5.17: Food group factor loadings for the four dietary patterns identified in the study population</td>
<td>137</td>
</tr>
<tr>
<td>20.</td>
<td>Table 5.18: Odds ratio and 95% CI for oral cancer, in approximate tertile for food groups defined by factor analysis</td>
<td>138</td>
</tr>
</tbody>
</table>
ABBREVIATION

bp : base pair
BMDP : BioMeDical Package (statistic software)
CYP : Cythocrome P450
DNA : Deoxyribonucleic acid
dNTPs : deoxyribonucleotide triphosphate
EDTA : Ethylene Diamine Tetraacetic Acid
FFQ : Food Frequency Questionaire
GSTM : Gluthathione S transferase mu
GSTT : Glutathione S transeferase tetha
KMO : Kaiser-Meyer-Olkin (Measure of Sampling Adequacy)
MgCL : Magnesium Chloride
mL : milliliter
mM : milliMolar
OR : Odds Ratio
PCR : Polymerase Chain Reaction
RFLP : Restriction Fragment Lenght Polymorphism
SCC : Squamous Cell Carcinoma
95% CI : 95 % Confidence Interval