ABSTRACT

Introduction: In the Malaysian population, the Indians were identified as having a higher oral cancer (OC) risk compared to Malays, Chinese and other ethnicities with betel quid chewing contributing the major factor among Indian female. Cancer progresses through a series of histopathological stages where the progression is thought to be driven by the accumulation of genetic alterations (Garnis et. al., 2009). Therefore, the identification of chromosomal alterations will not only enhance the understanding of the biology in this process, but will also identify important genes that might involve in oral carcinogenesis. Thus, this study utilised array Comparative Genomic Hybridization (arrayCGH) technology as a platform.

Aims: To identify chromosomal alterations leading to the identification of genes involved in the development of OC, and to determine the relationship between selected altered regions with sociodemographic characteristics which include gender, ethnicity and habits and selected clinicopathological parameters namely tumour nodes metastasis (TNM) stage, tumour size (T), lymph node status (N), tumour grade and tumour site.

Methods: A total of 20 fresh frozen tumour tissues diagnosed as OC and reference DNA from peripheral blood lymphocytes were obtained from the Malaysian Oral Cancer Data and Tissue Bank System (MOCDTBS)-Oral Cancer Research and Coordinating Centre (OCRCC), University of Malaya (UM). A series of 750 µm thick tissue sections were mounted in Optimal Cutting Temperature (OCT) compound and stained with H & E. Tissue sections containing more than 70% epithelial tumour were selected and confirmed by an oral pathologist. Analysis of arrayCGH was performed using the Human Genome CGH Microarray KIT 4x44K chip platform. Graphical

overview of chromosomal alterations was obtained by using the Genomic Workbench Standard Edition 5.0.14. Validation of *DUSP22* gene generated from arrayCGH results was then carried out at DNA (copy number) and cDNA (gene expression) levels. Statistical analysis was performed using SPSS version 19.

Results: In this study, the occurrence of deleted regions was slightly higher than the amplified regions**.** The most frequently deleted regions were detected on chromosome 19p13.3, followed by 19p13-p13.11 and 19q13.3 while amplifications were most commonly detected at 8q24.3, 8q11.1-8q11.2 and 3q26 regions. The most common amplified gene detected was *DUSP22*, followed by *KIAA0146*. For the deleted regions, the most common genes detected were *FBXO25*, *INPP5A*, *NKX6-2*, *C10orf92*, *CDH4*, *TAF4*, and *LSM14B*. With respect to the clinicopathological parameters (tumour size (T), TNM stage, lymph node status (N), tumour grade and tumour site) and sociodemographic profile (gender, ethnicity and habits), no association was found between the most amplified (8q24.3, 8q11.1-8q11.2 and 3q26) and deleted regions (19p13.3, 19p13-p13.11 and 19q13.3). Only region 19p13-p13.11 showed significant relationship (p=0.044) with tumour site. Validation and analysis at copy number (CN) and gene expression (GE) level for the *DUSP22* gene revealed consistent results with previous arrayCGH in which tumour cells showed over-expression.

Conclusion: This study showed no association between chromosomal alterations and the clinicopathological parameters and sociodemographic profile studied. Only tumour site showed correlation with chromosomal alteration. It is recommended that a bigger study be conducted using bigger sample size in order to obtain more significant association.

ABSTRAK

Pengenalan: Di kalangan masyarakat Malaysia, etnik india merupakan golongan yang berisiko tinggi menghidapi penyakit kanser mulut berbanding etnik Melayu, Cina dan kaum lain. Ini adalah disebabkan oleh tabiat mengunyah sireh terutamanya di kalangan wanita etnik India. Perkembangan kanser dari normal ke metastasis berlaku melalui siri peringkat histopatologi di mana perebakan kanser adalah disebabkan pleh pengumpulan aberasi genetik (Garnis et. al., 2009).. Oleh itu, pengenalpastian aberasi kromosom bukan sahaja meningkatkan pemahaman mengenai proses biologi yang berlaku dalam kanser mulut bahkan dapat mengenalpasti gen-gen penting yang mungkin terlibat dalam perkembangan kanser mulut. Sehubungan dengan itu, penyelidikan berlandaskan teknologi microarray Comparative Genomic Hybridization (arayCGH) dipilih sebagai platform utama penyelidikan ini.

Objektif: Untuk mengenalpasti aberasi kromosom dan gen-gen yang terlibat dalam perkembangan kanser mulut dan menentukan perkaitan di antara aberasi kromosom dengan ciri-ciri profil sosiodemografi serta parameter patologi klinikal tertentu termasuk peringkat TNM, saiz tumor (T) , status nodus (N), pengkelasan tumor dan kawasan penyebaran tumor.

Kaedah: 20 tisu tumor sejukbeku didiagnosis sebagai kanser mulut disaring daripada databank MOCDTBS-OCRCC UM. 750 µM tisu tumor sejukbeku dipotong dengan menggunakan cryostat dan diwarnakan dengan pewarnaan H & E. Diagnosis untuk sampel tisu melebihi 70% mengandungi epitelial tumor disahkan oleh pakar patologi mulut. Analisis arrayCGH dilakukan dengan menggunakan cip CGH Microarray KIT platform 4x44K. Grafik perubahan kromosom dianalisis dengan menggunakan perisian

Genomic Workbench Standard Edisi 5.0.14. Validasi gen *DUSP22* yang diperoleh dari arrayCGH dijalankan pada peringkat DNA ('copy number' (CN)) dan cDNA (pengekspresan gen (GE)). Analisis statistik dilakukan dengan menggunakan SPSS versi 19.

Hasil: Aberasi kromosom untuk delesi dikesan pada frekuensi yang tinggi pada kromosom 19p13.3, diikuti oleh 19p13-p13.11 dan 19q13.3 dan amplifikasi kromosom paling banyak dikesan pada kromosom 8q24.3, 8q11.1-8q11.2 dan 3q26. Sementara itu, amplifikasi gen *DUSP22* adalah yang paling kerap berlaku dalam kajian ini diikuti oleh gen *KIAA0146*. Sementara itu, untuk lokus delesi, gen yang paling banyak yang dikesan adalah *FBXO25, INPP5A, NKX6-2, C10orf92, CDH4, TAF4,* dan *LSM14B*. Berdasarkan kepada analisis statistik, tiada hubungan signifikan didapati di antara parameter patologi klinikal dengan aberasi kromosom yang paling kerap dikesan. Walau bagaimanapun, hanya lokus 19p13-p13.11 dikesan mempunyai hubungan yang signifikan di antara kawasan penyebaran tumor (p=0.044). Validasi dan analisis untuk gen DUSP22 pada peringkat CN dan GE mengesahkan bahawa sampel yang mengalami amplifikasi pada peringkat kajian arrayCGH juga mengalami over-expressed dan penggandaan kromosom.

Kesimpulan: Kajian ini menunjukkan bahawa tiada hubungan di antara aberasi kromosom dengan parameter patologi klinikal dan profil sosiodemografi. Hanya parameter kawasan penyebaran tumor dilihat mempunyai potensi untuk dikaitkan dengan prognosis awal kanser mulut. Kesimpulannya, adalah dicadangkan bahawa untuk kajian penyelidikan lebih lanjut, kajian terhadap aberasi kromosom harus dijalankan dalam skala saiz sampel yang besar, pengkhususan terhadap parameter

patologi klinikal yang lebih spesifik seperti lokasi tumor bagi mendapatkan hasil penyelidikan yang lebih signifikan.

ACKNOWLEDGEMENT

Bismillahirahmanirrahim, first of all, I would like to say Alhamdulillah, for giving me the strength and health throughout this research until its completion. This dissertation would not have been possible without the guidance and the help of several individuals who contributed and extended their valuable assistance in the preparation and completion of this study. First and foremost, I am heartily thankful to Professor Rosnah Mohd Zain and Professor Sharifah Noor Akmal Syed Husain, both my supervisors whose showed sincerity and encouragement that I will never forget. Their guidance and support from the initial to the final level enabled me to develop a better understanding of the study more thoroughly. Thanks Prof! Really appreciate it.

Not forgotten I would also like to express my utmost gratitude to my beloved husband, Abang Syahrizan Abang Slamsa and my family for their unfailing moral support and advice, who were always for me when I was having difficulties and gave me courage to finish this study. I was blessed with the advancement of internet technology, books, computers, as my sources to complete this project successfully.

Special thanks to Professor Dr Cheong Sok Ching, my colleagues and staff in Oral Cancer Research & Coordinating Centre (OCRCC), Diagnostic Laboratory of Oral Pathology, Oral Medicine and Periodontology (OPOMP), Cancer Research Initiatives Foundation (CARIF) and Molecular Cytogenetics Laboratory at Universiti Kebangsaan Malaysia Medical Centre (UKMMC), for their kind assistance, sharing ideas and guidance in the laboratory procedures and better understanding on oral cancer. Thanks all for your kind help! I also offer my regards and blessings to all of those who supported me in any respect during the completion of the study. Last but not the least,

above all of us, thanks to almighty Allah, for answering my prayers and giving me the strength to continue this study, thank you so much Dear Allah for the blessing. Alhamdulillah.

TABLE OF CONTENTS

4. MATERIALS AND METHODOLOGY

5. RESULTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX

LIST OF ABBREVIATIONS

WHO	World Health Organization
ICD	International Statistic Classification of Disease
NCR	National Cancer Registry
NIH	National Institutes for Health
OSCC	Oral Squamous Cell Carcinoma
FISH	Fluorescence in-situ Hybridization
CGH	Comparative Genomic Hybridization
arrayCGH	array Comparative Genomic Hybridization
GE	Gene Expression
SNP	Single Nucleotide Polymorphism
RT-qPCR	Real Time quantitative Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
gDNA	genomic Deoxyribonucleic acid
NCI	National Cancer Institute
ASR	Age Standardize Rate
CR	Crude Incidence Rate
GLOBOCAN	Estimation of the Burden of Cancer
IARC	International Agency for Research on Cancer
TNM	Tumour-Node-Metastasis
UICC	The International Union Against Cancer
AJCC	American Joint Committee on Cancer
UM	University of Malaya
HPV	Human Papilloma Virus
OC	Oral Cancer
BQ	Betel Quid
TSNA	Tobacco Specific Nitrosamines
ROS	Reactive Oxygen Species
USA	United States of America
mg/g	miligram/gram
β	Beta
ACS	American Cancer Society
GSTM1	Glutathione S-Transferase MU1

GSTT1	Glutathione S-Transferase Theta1
CYP1A1	Cytochrome P450,family 1,subfamily A,polypeptide 1
M	Mitosis
G1	Growth One
G2	Growth Two
S	Synthesis
RNA	Ribonucleic acid
TSG	Tumour Suppressor Gene
ONCO	Oncogene
GS	Growth Signal
bp	base pair
EGFR	Epidermal growth factor receptor
c-myc	Myelocytomatosis viral oncogene homolog
int-2	Integrator complex subunit 2
hst-1	Heparin-binding secretory transforming factor 1
PRAD-1/CCND1/ bcl-1	Cyclin D1
VEGF	Vascular endothelial growth factor
MMP	Matrix metalloproteinase
TGF	Transforming growth factor
Her-2	Verb erythroblastic leukemia viral oncogene homolog 2
Rb	Retinoblastoma
RUNX3	Runt-related transcription factor 3
PRTFDC1	Phosphoribosyl transferase domain containing 1
p53	p53 gene
LOH	Loss of heterozygosity
CDKN2A/p16	Cyclin-dependent kinase inhibitor 2A
FHIT	Fragile histidine triad
HGD	High Grade Dysplasia
CIS	Carcinoma in situ
MOCDTBS	Malaysian Oral Cancer Database and Tumour Bank System
OCRCC	Oral Cancer Research and Coordinating Centre

µm	micrometer
ml	mililiter
OCT	Optimal Cutting Temperature
°C	Celcius
rpm	revolutions per minute
µl	micro-liter
mg/mL	milligram per mililiter
mg/g	milligram per gram
TE	Tris EDTA
ng/µl	nanogram per microliter