CHARACTERISATION OF MUTATIONS IN EXONS 6 AND 7 OF THE TP53 GENE IN ORAL SQUAMOUS CELL CARCINOMA IN KENYA - A PILOT STUDY

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V60/71502/2009

THIS DISSERTATION IS SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE OF MASTER OF DENTAL SURGERY IN ORAL AND MAXILLOFACIAL SURGERY OF THE UNIVERSITY OF NAIROBI

DECLARATION

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DEDICATION

1. I dedicate this work to Carolin Averbeck for her support, guidance and patience during the course of my studies.

2. And to all head and neck cancer patients in the hope that research in this field will one day bring cure and restore joy to you and your families.

ACKNOWLEDGEMENTS

I am indebted to all my supervisors: Prof JF Onyango, Prof LW Gathece, Dr AW Njiru and Dr EAO Dimba for their indispensable guidance throughout this study. My sincere thanks also to Dr Isabella Oyier of the Centre for Biotechnology and Bioinformatics (CEBIB) of the University of Nairobi for her guidance on molecular aspects of this work. I am grateful to the Director of CEBIB for granting me permission to use the laboratory at CEBIB and making me feel welcome all the time. Special thanks to Ms Ann Owiti of CEBIB for all the hours spent in the laboratory taking me through the DNA procedures. I also thank Mr Ruben Yaa of CEBIB for helping me make sense of DNA sequencing. Special mention to Dr EAO Dimba and Mr Sigei of the University of Nairobi Dental Hospital (UoNDH) histopathology laboratory for all the help with preparation of paraffin blocks. I could never have done this study without you. Special thanks to Mr Desmond K'Owino and Mr Joshua Simba for making sense out of the raw data and for helping with statistical work. I also thank Mr. Jose Geda of Inqaba Biotechnical Industries (Pty) Ltd. for his help with primers and sequencing.

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KEY DEFINITIONS

1. Mutation – a change that occurs in the DNA sequence resulting from transcription errors during replication, exposure to ionizing radiation, exposure to mutagens, or viral infection
2. Germ line mutation – a mutation occurring in the eggs and sperm and can therefore be passed on to the offspring
3. Somatic mutation – mutations that occurs in body cells and are not passed on to offspring.
4. Polymorphism - refers to a DNA sequence variation that is common in the population, unlike a mutation which is deviation of a DNA sequence from normal. The most common type of variation is the single nucleotide polymorphism (SNP or snip), in which a single base differs between individual (being T instead of G, for example).
5. Gene amplification -refers to an increase in the number of copies of a gene. This is common in cancer cells, and some amplified genes may cause cancer to progress and/or become reisitant to cancer drugs. Gene amplification is also done in the laboratory by PCR for research purposes.
6. Exons – the DNA part where protein coding occurs by the processes of transcription and translation.
7. Introns – the part of DNA that does not directly code for proteins.

LIST OF ABBREVIATIONS

ARV	Antiretrovirals
CEBIB	Centre for Biotechnology and Bioinformatics
DBD	DNA binding domain
dH2O	Distilled water
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FFPE	Fresh frozen paraffin-embedded
GLOBOCAN	Global cancer statistics
GOF	Gain-of-function
HCL	Hydrochloric acid
HIV	Human Immunodeficiency Virus
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papillomavirus
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
KNH	Kenyatta National Hospital
LOF	Loss-of-function
MDM2	Murine double minute 2
MgCl	Magnesium chloride
NaCL	Sodium chloride
OSCC	Oral squamous cell carcinoma
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rpm	Rotations per minute
SDS	Sodium dodecyl sulfate
SNP	Single nucleotide polymorphism
SPSS	Statistical Product and Service Solutions
SSCP	Single-strand conformational polymorphism
TNM	Tumour, node, metastasis staging of malignancy
TP53	Tumour protein 53
UoN	University of Nairobi
UoNDH	University Of Nairobi Dental Hospital
UTR	Untranslated
U-V	Ultra Violet
v/v	Volume-volume
WHO	World Health Organisation

ABSTRACT

Background

Oral squamous cell carcinoma (OSCC) is the commonest type of oral cancer accounting for over 96% of all reported oral cancer cases. GLOBOCAN 2012 reports the prevalence of lip and oral cavity cancer in Kenya to be more than 7.1 cases per 100,000 population. This is one of the highest prevalence rates in Africa. In Kenya oral cancer is reported to account for 3.6% of all cancers, with a slight male predominance. Onyango and colleagues in 2004 reported that OSCC accounted for 95% of all oral cancer in Kenya.

Mutation or functional inactivation of the tumour suppressor gene TP53 is an almost universal feature of all human cancers. Mutant TP53 results in the coding of p53 protein that has lost its tumour suppressive properties thus allowing damaged DNA to progress through the cell cycle unchecked. The end result is continued propagation of cancerous cells. Prevalence rates of TP53 mutations have been reported from 16-96% while the International Agency for Research on Cancer (IARC) puts the prevalence at 42%. The commonest mutation type reported is the missense mutation at 75% of all mutations. Over 200 single nucleotide polymorphisms (SNPs) have been reported in literature. Only 1 SNP has been reported in the DNA binding domain (DBD).

Study objective

The broad objective was to characterise the mutations in exons 6 and 7 of TP53 gene in OSCC reported at a university teaching hospital in Nairobi, Kenya.

Study design

This was a cross-sectional descriptive laboratory based study including all OSCC reported and archived as paraffin-embedded tissue blocks in the years 2012 and 2013.

Study setting

The histopathology laboratory at the University of Nairobi Dental Hospital (UoNDH) provided paraffin-embedded tissue blocks for DNA extraction while DNA analysis for mutations was carried out at the molecular laboratory of the University of Nairobi Centre for Biotechnology and Bioinformatics (CEBIB). DNA sequencing was done at Inqaba Biotechnical Industries (Pty) Ltd.

Sampling method

A non-probability convenient sampling method of all cases of OSCC archived in 2012 and 2013 and that met the inclusion criteria were incorporated in the study.

Data collection procedures

Paraffin-embedded tissue blocks with a diagnosis of OSCC were collected from the histopathology laboratories and data regarding patient demographics, ethnicity/hospital of origin as well as tumour characteristics was tabulated. Additional clinical data was collected from patient files. At the Center for Biotechnology and Bioinformatics, DNA extraction was then carried out to yield clean DNA for analysis and PCR-SSCP procedures done to amplify DNA for sequencing. DNA sequencing was done at Inqaba Ltd. Mutation analysis was then done to identify mutations in exons 6 and 7 of the TP53 gene. The standard procedures recommended by the International Agency for Research on Cancer were followed. All the laboratory machines were calibrated and DNA extraction, amplification and analysis were done with the help of a qualified molecular technologist.

Results

The male to female ratio was 1:1 though the females (mean age 59.49 years) were older than the males (mean age 53.31 years). The commonest complaint at presentation was an ulcer 107/157 (75.9%) while the commonest tumour site was the tongue 54/157 (35.3%). A majority of tumours were well differentiated 74/157 (48.4%) followed by poorly differentiated 65/157 (42.7%).

A total of 20 samples were analysed for TP53 mutations in exons 6 and 7 and 19 out of the 20 samples yielded good quality nucleotides for sequencing analysis. None of the samples showed TP53 gene mutations (0% prevalence). However, we found 6 single nucleotide polymorphisms

(SNPs) in codon 220 (5 SNPs) and codon 254 (1 SNP). These SNPs were in the introns in the DNA-binding domain (DBD) and were C→A (5 SNPs) leading to codon 220 Glutamate/Histidine substitution and G→T (1 SNP) resulting in codon 254 Cysteine/Glycine substitution. More significantly is that these SNPs have not been reported previously while it is only the second report of SNPs in the DBD.

1.0 CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Oral squamous cell carcinoma (OSCC) is the commonest type of oral cancer accounting for over 96% of all oral cancer case[1] The World Health Organisation (WHO) ranks OSCC as the 6th commonest cancer among males and 10th among females[2]. The health burden from OSCC is increasing especially in the developing world where oral health resources are scarce and often overstretched. The average worldwide incidence of OSCC is estimated to be 6.6 cases per 100,000 men and 2.9 cases per 100,000 women, with worldwide estimates of about 600,000 cases of head and neck squamous carcinoma (HNSCC) in 2011[2]. Of these patients, only 40-50% are expected to survive beyond 5 years. Oral cancer thus constitutes a major public health problem worldwide. The scarcity of health resources in a developing country like Kenya coupled with late presentation of oral cancer patients has further compounded the problem.

1.2 Worldwide and Kenyan cancer statistics

More than half (57%, n = 8 million) of new cancer cases and nearly two thirds of related deaths (65%, n = 5.3 million) occur in the world's less developed regions[1, 2]. Many developing countries are going through rapid societal and economic changes, and there is a shift toward lifestyles representative of industrialised countries. These factors, along with changes in reproductive, dietary, and hormonal risk factors, are contributing to the rising cancer rates[1].

OSCC accounts for approximately 96% of all oral cancers and it is the 6thmost common cancer among males and 10th among females[2]. The prevalence of lip and oral cavity cancer in Kenya is more than 7.1

cases in 100,000 people according to the statistics for Africa (Figure 1)[2]. In Kenya oral cancer is reported to account for 3.6% of all cancers, with a slight male predominance (M: F = 1.3:1) and OSCC accounts for 95% of all oral cancers in Kenya[3].

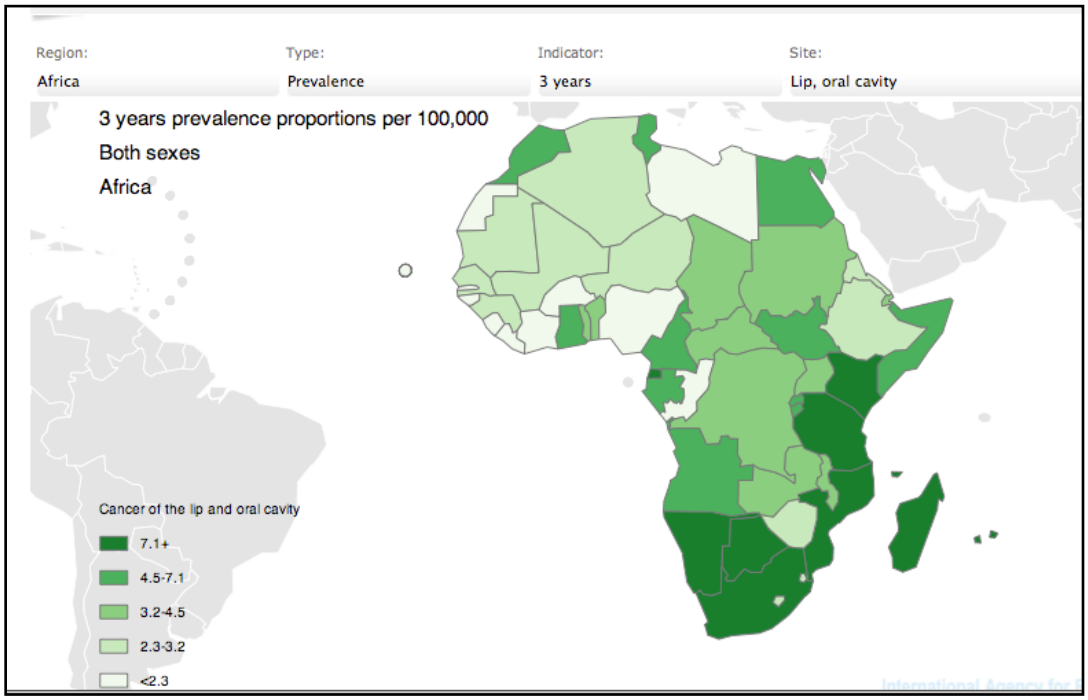

Figure 1 Cancer statistics map for the African continent

Source: GLOBOCAN-IARC, 2012. Open source

1.3 The role of TP53

TP53 is the gene which codes for the expression of the p53 protein, a stress-response protein that functions mainly as a transcription factor. The p53 protein mediates changes in gene expression leading to senescence, cell cycle arrest, or apoptosis. The net effect of p53 expression would be to allow DNA repair or the elimination of severely damaged cells and in this way suppress tumour development. Physiological levels of p53 in healthy cells are very low while mutant p53 is highly accumulated in cancer cells[4, 5].

Mutant p53 loses its wild-type functions that would normally direct damaged cells to undergo growth arrest. Therefore, mutant p53 leads to aberrant cell proliferation because the cells cannot regulate cell division and growth. Cells in which mutant p53 has accumulated do not undergo apoptosis following DNA damage. Such cells continue to grow uncontrollably leading to development of tumours[4-7].

About 50% of all human tumours have been shown to have mutations in tumour protein 53 (TP53) gene[1, 2]. The International Agency for Research on Cancer (IARC) database shows approximately 20,000 described mutations in this gene. Up to 75% of the mutations[1, 2, 4, 5], are of the missense type and occur at over 200 codons within the DNA binding domain (DBD) of the gene.

1.4 The structure of TP53 gene and p53 protein

The TP53 gene is located on chromosome 17 (17p13). It has 11 exons. The p53 protein consists of 393 amino acids with five functional domains: two N-terminal trans-activation domains followed by a proline-rich domain, then a central DBD and a C-terminal tetramerization domain[5].

1.5 TP53 mutations

1.5.1 Localisation of mutations

Most mutations are known to occur in the DBD with the commonest mutations localised to a few nucleotides in the DBD[5, 8, 9]. In OSCC 98% of all p53 mutations have been localised to exons 5-8[7, 10]. Mutations in the DBD in turn weaken the binding of p53 to DNA by causing structural destabilisation of the DBD. Failure of p53 to bind DNA means that defective cells continue to divide and grow thus leading to cancer development.

1.5.2 Types of mutations and polymorphisms

Mutations are changes in an organism's genetic material which may be due errors in replication, transcription errors, radiation, viruses and many other factors. Mutations may be classified according to the change that occurs in the nucleotides/base pairs into:

1. Base Substitutions – a single nucleotide is exchanged for another.
2. Base Insertion– extra base pairs are inserted leading to a nonsense mutation.
3. Base Deletions – some base pairs are deleted/lost. Also leads to a nonsense mutation.
4. Base inversions **–** two base pairs or more are inverted in their postion.
5. Polymorphism **–** refers to a DNA sequence variation that is common in the population, unlike a mutation which is deviation of a DNA sequence from normal. The most common type of variation is the single nucleotide polymorphism (SNP or snip), in which a single base differs between individual (being T instead of G, for example).

Mutations are also classified as either disruptive mutations or non-disruptive mutations. Disruptive mutations are non-conservative and occur within the DBD and stop codons while non-disruptive mutations are termed conservative as they occur away from the DBD and the stop codons[4, 9, 11]. The type of mutation has been shown to impact on tumour characteristics; disruptive mutations, for example nonsense mutations, have been shown to decrease overall patient survival[7]. The cumulative incidence of deaths from head and neck squamous cell carcinoma has been shown to be higher among patients with mutant TP53 than in those with wild-type TP53. This incidence is particularly highest in those patients with disruptive mutations[7].

1.5.3 Methods of TP53 mutation analysis

There are many methods available to study TP53 alterations. These include immunohistochemistry, functional testing by use of yeast and mutational analysis using Polymerase Chain Reaction (PCR) and DNA sequencing. Immunohistochemistry yields inconsistent results and fails to detect various types of mutations. For this reason the use of rapid and sensitive mutation analysis by DNA sequencing and PCR is currently favoured[4, 5, 12]. Most authors have limited their mutation analysis to the DBD, specifically exons 5-8.

1.5.4 The need for TP53 mutation analysis

Despite advances in health care in general and molecular cancer genetics specifically, the 5-year survival rate for OSCC patients remains 40-50%[2, 7]. It is hoped that with further understanding of the molecular basis of OSCC and cancer in general, the end result would be novel therapies that are tailored to the individual patient and, therefore, improved patient survival. Oncologists want to know the TP53 mutations as these may influence the choice of chemotherapy – patients with a tumour that bears a TP53 mutation would receive one treatment while those without TP53 mutation receive different treatment. In addition, small molecules that specifically target mutant TP53 using retroviral and adenoviral vectors are under development. This approach would be specific as it would target only cancer cells since only they have mutant TP53.

This study was meant to study the clinic-pathological characteristics of OSCC and to characterise the TP53 mutations found in these OSCC patients diagnosed at a tertiary centre in Nairobi, Kenya.

1.5.5 Mutations in TP53 and OSCC aetiology

The most important risk factors for OSCC are tobacco use, alcohol, betel-quid, as well as infection with high-risk human papillomavirus (HPV) genotypes. These risk factors are known to be mutagenic. The mutagenic effects are dependent upon dose, frequency of use, and duration of use. However, not all persons who have the above risk factors develop OSCC and not all persons with OSCC have identifiable risk factors. This, therefore, may point at the possible presence of person-specific genetic characteristics that either provide protection against the development of OSCC or predispose to the development of OSCC[7].

Mutation or functional inactivation of the tumour suppressor gene p53 is an almost universal feature of all human cancers[4-6, 9]. Normal p53 results in optimal function of cell mechanisms including apoptosis, cell senescence and cell cycle arrest that eliminate damaged cells and suppress tumour growth. In normal cells, the p53 protein level is low. DNA damage triggers the increase of p53 proteins which in turn may leads to arrest of cell growth. The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. A cell can arrest its progression through the cell cycle at a number of checkpoints following the detection of DNA damage.

TP53 has been associated with delays in transit through both G1 and G2 checkpoints, as well as in the mitotic spindle checkpoint. Cell cycle arrest is, therefore, a prominent outcome of DNA damage and is induced in many cell types by the expression of wild-type p53. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA[5, 7].

The most common mutation identified in TP53 gene is the missense mutation in which a single nucleotide is substituted by another resulting in a full-length protein containing only a single substituted amino acid[4, 9]. Other mutation types that have been identified include nonsense mutations, deletions,

insertions and inversions. These mutations result in a truncated protein or complete absence of protein synthesis. The mutation type would, therefore, impact on tumour development and behaviour; missense mutations producing a complete, but, abnormal protein are generally expected to have less functional disruption than a deletion that completely stops protein synthesis.

TP53 mutations have been shown to be diverse in their location on the p53 coding sequence but are distributed in all coding exons of the TP53 gene, with a strong predominance on exons 5-8[4]. The exons 5-8 constitute the DBD of the protein, and it has been widely studied in relation to TP53 gene mutation.

1.5.6 TP53 single nucleotide polymorphisms and OSCC

Besides mutations, polymorphisms of TP53 have also been reported as possible risk factors for a number of tumours. Over 200 SNPs have been reported in the TP53 gene with the majority occurring in the exons. The most widely studied SNP is the Pro72Arg at codon 72 of the TP53 gene[13-15]. This SNP at codon 72 of TP53 (Pro72Arg) results in two protein forms with different biological and bio-chemical properties [13]. In particular, the Arg72 variant induces markedly better apoptosis compared to the Pro72 variant, and these two functionally distinct polymorphic variants of TP53 may influence cancer risk or treatment[13]. There have been conflicting reports on the clinical relevance of single nucleotide polymorphisms (SNPs) on oral cancer progression and response to therapy. The role of SNPs in tumour biology is still under research with only about 200 SNPs reported in literature.

1.5.7 Wide variations in reported literature on prevalence of TP53 mutations

The International Agency for Research on Cancer (IARC) reports the prevalence of TP53 somatic mutations in head and neck cancer to be 42%[8, 12]. However, published studies have reported a wide variation in the prevalence of TP53 mutations ranging from 16%[16] to 93%[17]. These wide variations in

reported mutations have been attributed to differences in methods of gene analysis, differences in sample sizes of different studies, and even geographical variations [12].

A study of TP53 mutations in 50 head and neck squamous cell carcinoma using peripheral blood and tumour tissue in Polish patients found that only 8 out of 50 cases (16%) showed TP53 mutation[16]. The study found no correlation between the TNM tumour stage and TP53 mutations. In addition, there was no statistically significant association between tumour site and histological differentiation. This study appears to imply that p53 mutations have no relation to tumour characteristic, which is in contrast to what is known about the impact of p53 mutations and their influence on tumour behaviour.

In another study by Poeta et al. (2007), 560 patients with head and neck squamous cell carcinoma were analysed for TP53 mutations and 224 out of 420 patients (53.3%) had TP53 mutations where the presence of any type of mutation was shown to have been associated with decreased overall survival. These findings indicate that the presence of TP53 mutations impacts negatively on prognosis and may actually be used as a prognostic marker.

A study of 46 HNSCC in Finland reported a prevalence of 26/46 (57%) and poor prognosis as well as poor response to radiotherapy when mutations occurred in the DBD of the TP53 gene[18]. This study thus agrees with that of Poeta (2007) that showed the prognostic significance of TP53 mutations[7]. This goes further to show that the presence of TP53 mutations confers some degree of radio-resistance thus further impacting on prognosis.

In a Sudanese study among 14 tobacco snuff users and 14 non-tobacco snuff users with OSCC, 13 out 14 (93%) snuffers had TP53 mutations while 8 out of 14 (57%) non-tobacco users (controls) also showed mutations[19]. This shows that p53 mutations are influenced by tobacco which is a known mutagen in OSCC carcinogenesis.

A Taiwanese study examined 187 specimens of OSCC among betel quid chewers and cigarette smokers for TP53 mutations in exons 5-9 and found mutations in 91 out of 187 cases (49%) and reported no association with age, sex,TNM stage, status of cigarette smoking or betel quid chewing[20]. This study, therefore, disagrees with others who have correlated p53 mutations and tumour characteristics as well as the influence of tobacco use on mutations.

Sakai et al.(1999) [17] analysed 15 tumour tissues with OSCC and found mutations in 14 out of 15 cases (93%) localised to the region of exon 5-8. This was a small study in terms of sample size which could explain the very high percentage of mutations reported when compared to other studies.

According to a study by Oliver and colleagues (2004) [5], the spectrum of TP53 mutations correlates with carcinogens and risk factors. Missense mutations, accounting for about 75%, implicate environmental carcinogens in OSCC. The other 25% mutations were nonsense, frame-shift mutations and deletions. These findings tally with the fact that environmental carcinogens lead to missense mutations and appear to explain why the majority of p53 mutations are missense: Environmental influences play a large role in OSCC tumourigenesis.

1.5.8 Controversies in the literature

A wide review of the available literature, therefore, appears not to agree on three issues. First, there is a huge variation in the reported incidences of TP53 mutations[12, 16, 17]. This may be explained by differences in sample sizes, methods used in gene analysis, and geographic variations. Secondly, whereas some have found mutations to impact on tumour characteristics, other studies have failed to show any such influence [7, 18, 20]. Lastly, there are divergent views on the influence of SNPs on mechanisms of TP53 gene function and their role if any in carcinogenesis[13-15].

1.6 Study justification

Studies on TP53 mutations and polymorphisms have displayed varied patterns of prevalence as well as wide geographical variations. Ethnic differences have also been reported in the literature[10, 13, 21, 22]. However, due to the paucity of studies from the East African region the comparison of mutations in this region with those from other geographic locations is not possible. It is this knowledge gap that this study sought to fill.

Most of the existing studies have been carried out in Europe and Asia, while there is limited data available on TP53 mutations in Africa in general and East Africa specifically. This study is the first attempt at studying TP53 mutations in a Kenyan population. TP53 mutation patterns show geographic variations between regions of high and low incidence of oral cancer, suggesting a role for region-specific factors[16, 20, 22-24].

1.7 Study objectives

1.7.1 Broad objective

To establish the prevalence and pattern of the mutations in TP53 gene of OSCC cases diagnosed at a university teaching hospital in Nairobi, Kenya.

1.7.2 Specific objectives

1. To analyse the demographic characteristics of OSCC among cases diagnosed in a histopathology laboratory in a university teaching hospital.

2. To describe the pattern of OSCC among cases diagnosed in a histopatholgy laboratory in a university teaching hospital.

3. To determine the prevalence of mutations in exons 6 and 7 as well as polymorphisms of the TP53 gene in OSCC from laboratory archival tissue blocks.

4. To analyse the pattern of these mutations and polymorphisms in OSCC based on archived laboratory tissue blocks.

1.8 Variables

The study variables were:

Independent variables	**Measure**
Age	Years
Gender	Male or Female
Ethnicity	Ethnic groups
Hospital	Referring hospitals
Tumour site	Location in oral cavity
Dependent variables	
Location of TP53 mutation	Exons6 or 7
Type of TP53 mutation	Missense, Nonsense
Type of polymorphism	SNP

2.0 CHAPTER 2 - MATERIALS AND METHODOLOGY

2.1 Study design

This was a cross-sectional descriptive clinico-laboratory study of OSCC and TP53 mutations in OSCC confirmed biopsy tissues at University of Nairobi Dental Hospital (UoNDH) diagnosed and archived in the years 2012 and 2013.

2.2 Study area

The UoNDH is the one of only two dental schools in Kenya offering training leading to qualification as a dental surgeon. It is located in Nairobi and has a fully functional histopathology laboratory that functions as a referral center for head and neck pathology. This histopathology laboratory receives biopsy specimens from the university's maxillofacial surgery clinic as well as from hospitals and clinics all over Kenya. This means that good representative samples of the OSCC cases seen in the greater part of Kenya are diagnosed in this laboratory.

2.3 Study population

OSCC histologically confirmed and paraffin-embedded tissue blocks were used as samples from which DNA was extracted. Clinical data was extracted from the files of patients who had histological specimens sent to the UoNDH for histopathology. The paraffin-embedded tissue blocks were collected from the histopathology laboratory at the UoNDH. On average the laboratory processes approximately 50-100 OSCC specimens annually. DNA samples were then extracted from paraffin-embedded tissue samples for TP53 mutation analysis by PCR.

2.4 Inclusion criteria

All OSCC histopathologically diagnosed tissue blocks for the two years 2012 and 2013 were included in this study.

2.5 Exclusion criteria

1. Tumour tissue in which OSCC was not the primary tumour was excluded.

2. Exclusion was made for specimens with incomplete data.

3. Tissue blocks from which inadequate DNA was extracted were also excluded.

2.6 Sampling method

All the tumuor samples that were diagnosed at the UoNDH histopathology laboratory in the years 2012 and 2013 were included in the study. A total of 157 samples were included. DNA analysis was done on a final random sample of 20 specimens to detect and analyse TP53 mutations.

2.7 Data collection technique and procedures

Data collection was done in three stages. The first stage involved collection of data of OSCC confirmed paraffin-embedded tissue blocks from the histopathology laboratory at UoNDH. These included hospital/laboratory number, age, gender, tribe, hospital, tumour site and histological differentiation.

The tumour blocks were then re-examined under a microscope by a qualified oral pathologist and the specific tumour regions marked (Figure 2). This was to ensure that DNA extraction was done specifically from the part of the block which had tumour tissue.

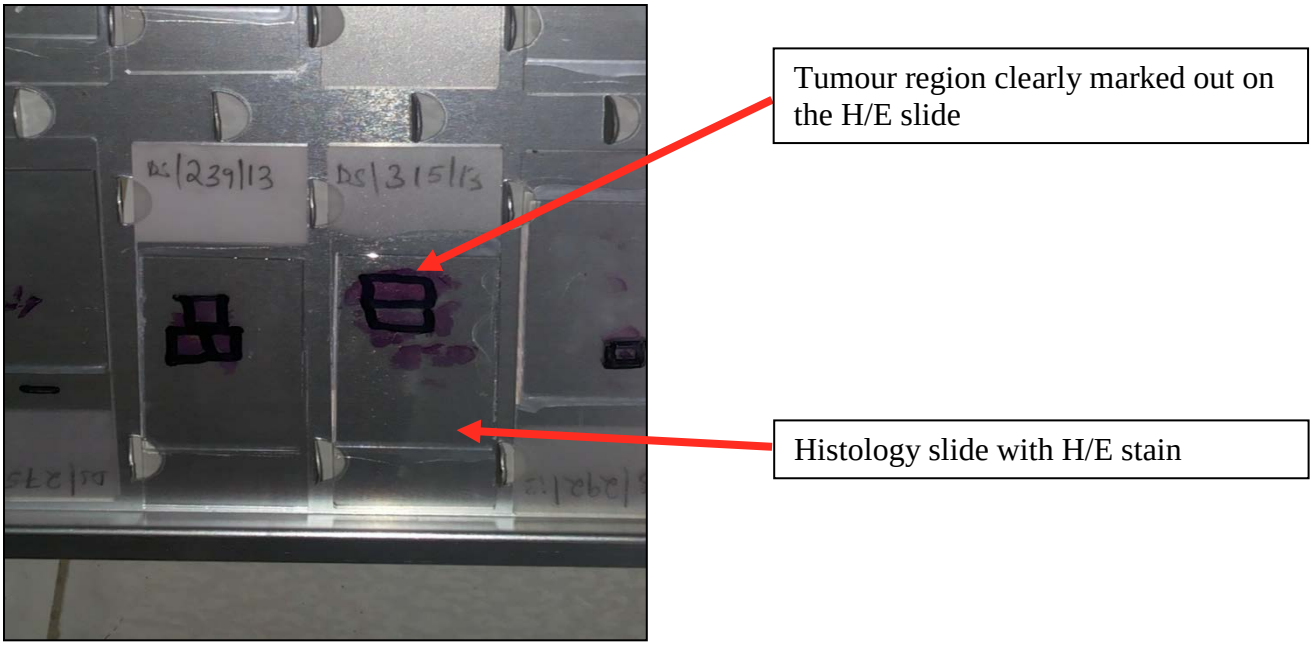

Figure 2 Tumour regions marked out on the H&E slides

Further data was collected from the clinical records of the patients in whom complete information was provided in the histopathology request forms. This involved getting patient files from the hospitals where these patients' biopsies had been done. We collected clinical data including tumor site, TNM staging, HIV status, imaging modalities at diagnosis, treatment provided as well as follow up periods.

In the second stage, the paraffin-embedded tissue blocks were transferred to the molecular laboratory of the Centre for Biotechnology and Bioinformatics (CEBIB) for DNA extraction, amplification and purification. DNA extraction from paraffin-embedded blocks was done using an extraction kit, blackPREP FFPE DNA Kit (Figure 3) (Analytik Jena, Germany). The DNA extraction procedure from the tissue blocks is detailed in Appendix A. Primers for the PCR amplification products were customised at Inqaba Biotechnical Industries (Pty) Ltd. in South Africa and are outlined in Appendix B. The Taq DNA polymerase reagents for constituting the PCR mix were procured from Qiagen USA and Promega UK.

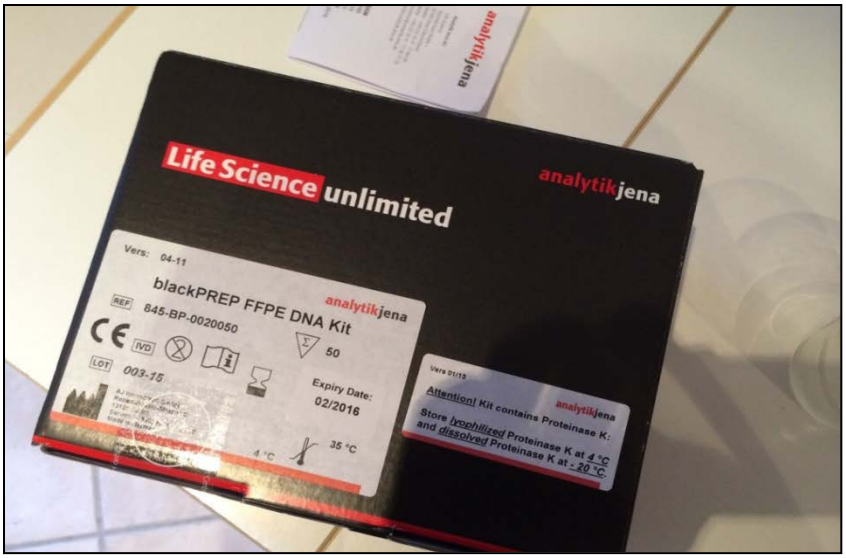

Figure 3 DNA extraction kit for formalin fixed paraffin-embedded (FFPE) tissue

The final DNA products (figure 4) were purified for sequencing using the Double Pure PCR Purification kit (Analytik Jena, Germany) and stored at -20ºC awaiting sequencing.

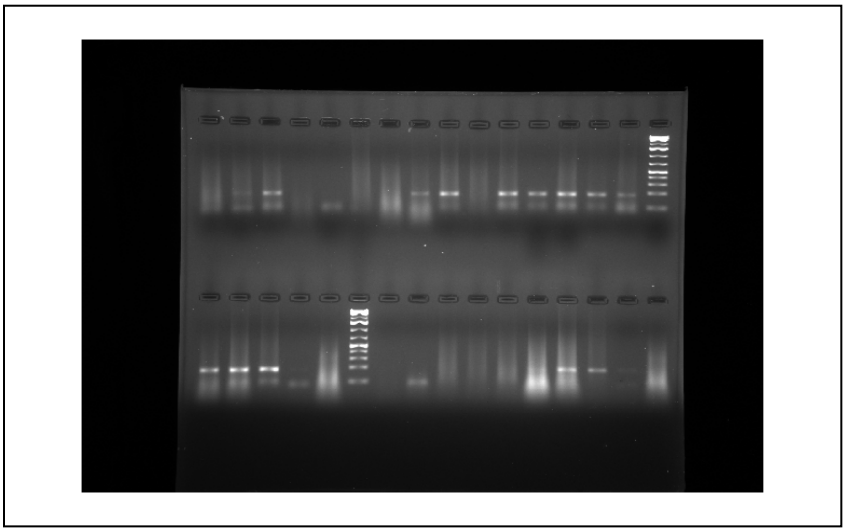

Figure 4 DNA gel showing the purified bands ready for sequencing

The procedures for PCR analysis including PCR conditions, reagents, and primers used is available in Appendix B. Both the DNA extraction and PCR amplification procedures were carried out at the molecular laboratory at CEBIB.

The third and final stage of data collection involved shipping of the amplicon to Inqaba Biotechnical Industries (Pty) Ltd. in South Africa for DNA sequencing. DNA sequencing was done in both the forward (5' to 3') and reverse (3' to 5') directions in order to capture the entire nucleotide sequence in the sequenced DNA. DNA sequencing and analysis was done on exons 6 and 7 including adjacent introns. The lengths of PCR fragments for our SSCP analysis were in the range of 135–245 bp and the sensitivity and specificity of this technique to detect mutations even if only present in a low amount is more than 90%[5]. Once the sequencing results were received the assembly and alignment of nucleotides to enable mutation analysis was done using three different software systems; Mega, UGene and Sequencher. These software systems enabled quick and precise analysis of mutations as well as generation of the charts and chromographs for presentation of results.

2.8 Validation and minimisation of errors

(i) Only those specimens which met the laid down inclusion criteria were included in this study.

(ii) The collection of the paraffin–embedded blocks was done only by the principal investigator.

(iii) There was strict application of the laid down protocols of DNA extraction, amplification and analysis.

(iv) The manufacturer's instructions in all the procured kits were strictly followed.

(v) All laboratory equipment had valid calibration certificates.

(vi) The principal investigator collaborated with molecular biologists at CEBIB who helped with the DNA procedures.

2.9 Data management and presentation

In the first part of data collection, a data collection form (Appendix C) developed by the investigator was used and all data entered into a computer package for data cleaning. Data was then tabulated and analysed using IBM SPSS Statistics version 20.

The final data on DNA extracted and ready for sequencing was entered into a standard laboratory form for shipping to South Africa. This is shown in Appendix D.

Finally the results of DNA sequencing results were analysed using Mega, UGene and Sequencher softwares all available at CEBIB.

The results are presented in the form of pie charts, bar graphs, and chromatographs.

2.10 Ethical considerations

The study protocol was approved by the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (Appendix E). All data collected regarding patients was kept confidential and coding was done during data entry so that names of patients did not appear in the data collection sheets. Relevant permissions were granted by the heads of the laboratories used in this study.

2.11 Competing interests

None

3.0 CHAPTER 3 - RESULTS

3.1 Socio-demographic data

In the initial phase a total of 157 patients were included in the study for the years 2013 and 2014. Of these 80 (51%) were male and 77 (49%) were females. The mean age was 57.07 ± 15.92 SD years. The females were older (mean 59.49 ± 16.26 SD) than males (mean 53.31 ± 15.08 SD). The difference was statistically significant (t=2.34, P=0.02). The age and gender distribution is as shown below in Figure 5.

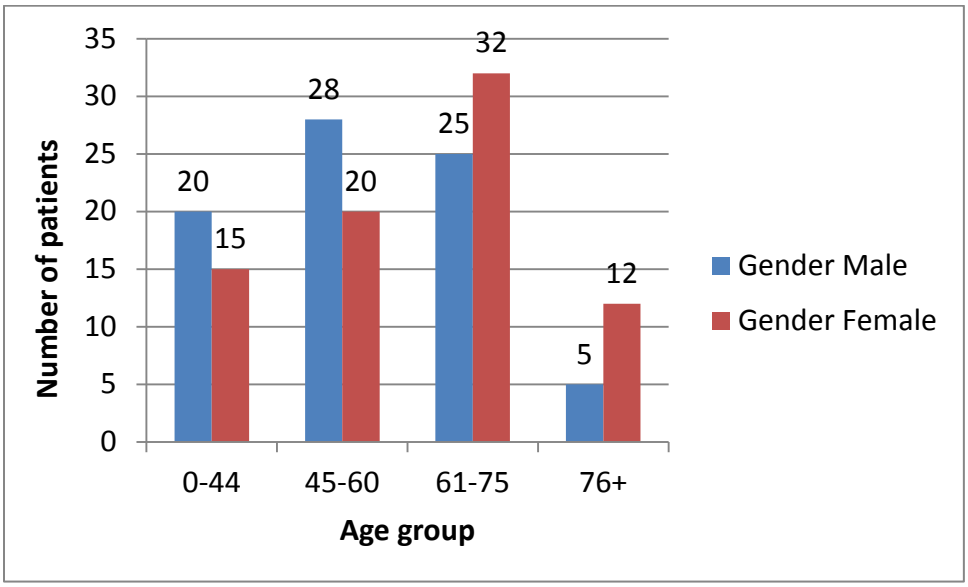

Figure 5 Distribution of patients by age and gender

The majority of patients 54 (34.4%) were referred from Meru Level V Hospital followed by those from UoNDH 42 (26.8%). The least number of patients 6(3.8%) were from KNH. The distribution of patients by referral hospital is shown in figure 6

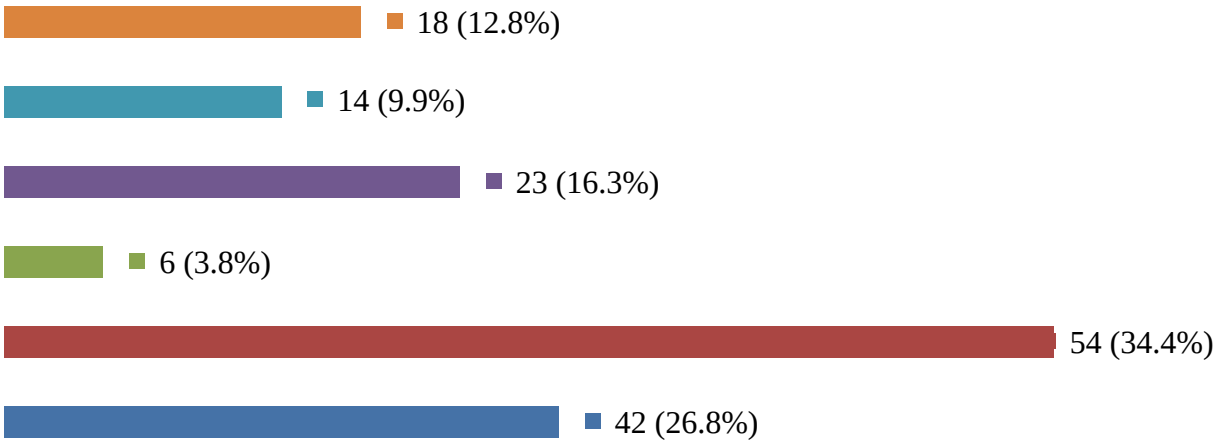

Figure 6 Distribution of patients by referring hospital

The gender distribution by hospital was not statistically significant (X^2=3.20, p=0.67). The patients from Nyeri Hospital were older (mean age 63.06 ± 16.79 years) while the patients from KNH were the youngest (mean age 52.40 ± 12.90 years). The mean age at presentation at the various hospitals was not statistically significant (F=0.77, p=0.58). The mean age and gender distribution of patients from various hospitals is shown in table 1.

HOSPITAL	MEAN AGE	MALE	FEMALE	TOTALS
MERU	53.25 ± 16.50	31(57.4%)	23(42.6%)	54(34.4%)
SDS	55.93 ± 12.81	21(50.0%)	21(50.0%)	42(26.8%)
MACHAKOS	58.68 ± 20.39	11(47.8%)	12(52.2%)	23(16.3%)
NYERI	63.06 ± 16.79	10(55.6%)	8(44.4%)	18(12.8%)
KNH	52.40± 12.90	2(33.3%)	4(66.7%)	6(3.8%)
PRIVATE	58.15± 12.62	5(35.7%)	9(64.3%)	14(9.9%)
TOTALS		**80(51.0%)**	**77(49.0%**	**157(100%)**

Table 1 Distribution of patients from referring hospitals by mean age and gender

In terms of ethnicity, the Meru had the highest number of OSCC patients 51(36.2%) followed by Kikuyu 44(31.2%), Kamba 24 (17.0%), Luo 5(3.5%), and others 10(7.1%). The Meru ethnic community had a higher percentage 16(31.4%) of younger patients (0-44 years) with diagnosis of OSCC more than any other ethnic group. This was followed by the Kikuyu with 5(11.4%). The distribution of age at diagnosis according to ethnic group is as shown in table 3. The age distribution by ethnicity was not statistically significant.

	ETHNIC GROUP					
AGE	**MERU**	**KIKUYU**	**SOMALI**	**LUO**	**KAMBA**	**OTHERS**
0-44	16(29.6%)	8(15.1%)	4(50.0%)	3(50.0%)	4(16.0%)	2(18.2%
45-60	15(27.8%)	21(39.6%)	1(12.5%)	1(16.7%)	7(28.0%)	5(45.5%
61-75	19(35.2%)	18(34.0%)	2(25.0%)	2(33.3%)	12(48.0%)	3(27.2%)
76+	4(7.4%)	6(11.3%)	2(25.0%)	0(0.0%)	2(8.0%)	1(9.1%)
TOTAL	**54(34.4%)**	**53(33.8%)**	**8(5.1%)**	**6(3.8%)**	**25(15.9%)**	**11(7.0%)**

Table 2 Age distribution by ethnicity

3.3 Clinicopathological characteristics

3.3.1 Presenting complaint

The most common complaint at presentation was an ulcer 107 (75.9%), followed by swelling 24 (17.0%), non-healing socket 7 (5.0%), pain and bleeding 3 (2.1%). The presenting complaint was not indicated in 16/157 (10.2%) of specimens and these are excluded in the analysis for presenting

complaint. The duration of presenting complaint varied from less than 2 weeks to over one year with most patients 42(51.5%) presenting 1 month to 6 months after the onset of symptoms.

3.3.2 Tumour site

The tongue was the commonest tumor site 54 (35.3%) followed by the buccal mucosa 30 (19.6%), hard palate 20(13.1%), maxilla 16 (10.5%), mandible 14 (9.2%), lower lip 14 (9.2%), other sites 4 (3.4%) (Figure7). There were 4 specimens in which tumour site was not indicated and these were excluded from site analysis.

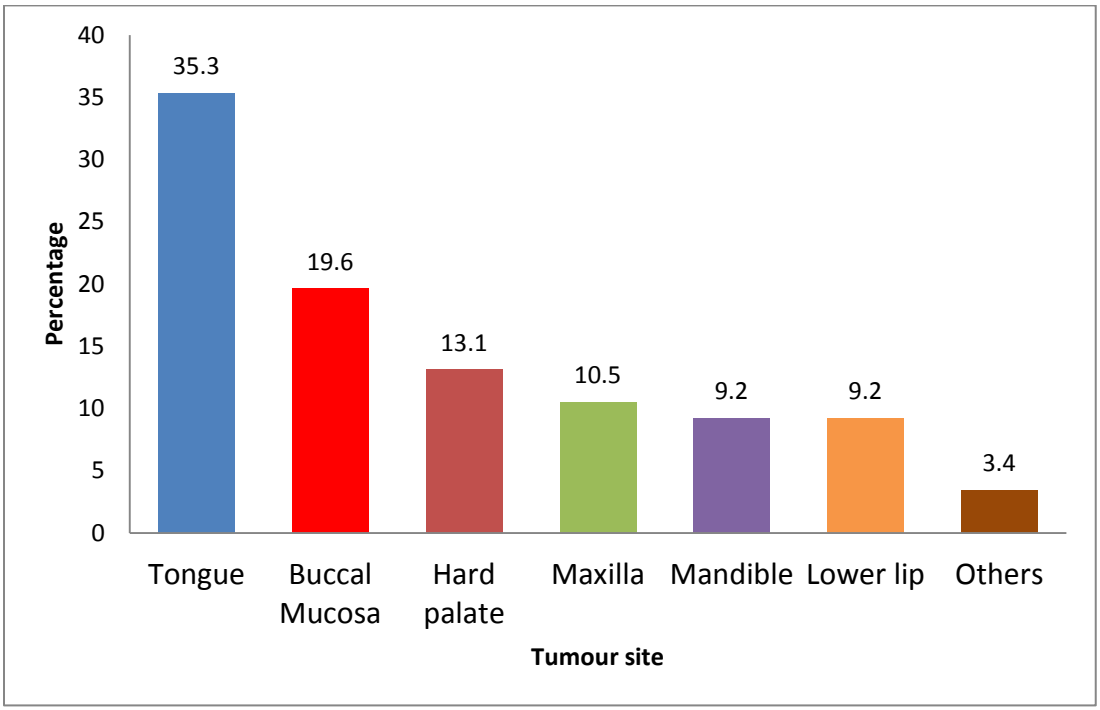

Figure 7: Tumour distribution by site

3.3.3 TNM Staging

We found that TNM stage was not indicated in a majority of cases 126/157 (80.3%). Of the 31/157 (19.7%) who had TNM staging indicated, 1(3.2%) was Stage II, 9 (29.1%) were in Stage III and 21 (67.7%) were in Stage IV. There were no patients in Stage I TNM classification.

3.3.4 Imaging modalities used

Imaging was done routinely with an Orthopantomogram (OPG) being the commonest at 45/157 (28.7%). A majority of those who had an OPG done (15/45, 33.3%) were attending clinics at the UoNDH. CT scan imaging was the next most common imaging modality 20/157(12.7%) while only 16/157 (10.2%) had a chest radiograph done to check for lung metastasis.

3.3.5 HIV Status

A majority of patients 125/157 (79.6%) either did not know their HIV status or it was not routinely done. Of the 32/157 (20.4%) in whom the HIV status was indicated, 26/32 (16.6%) were positive with 4/26 (2.5%) being already on anti-retroviral treatment. It was not clear why HIV testing was unknown or not done for most of the patients. None of the patients were tested for HPV despite the now known association between HPV and OSCC.

3.3.6 Treatment modalities

This study also tried to establish from the patient records what treatment modality, if any, was done following a diagnosis of OSCC. In total only 56/157 (35.7%) had complete information regarding follow up after diagnosis. Of these 15/56 (26.8%) had surgery alone, 10/56 (17.9%) had surgery combined with radiotherapy or chemo-radiotherapy while 21/56 (37.5%) were referred to palliative/hospice care. A majority of patients 132/156 (84.6%) did not have a record of treatment provided or were simply lost to follow up. The mean duration from the time patients presented to the clinics to the time treatment was

offered was 6 months; though a majority of patients 101/157 (64.3%) were either lost to follow up after a diagnosis was made or no record of the treatment offered was available.

3.3.7 Histological differentiation

The majority of OSCC reported were well differentiated 74 (48.4%) followed by poorly differentiated with 65(42.7%). Only 11 (7.0%) of the OSCC were moderately well differentiated. There were 2 recurrences (1 tongue and 1 maxilla) and 1 metastatic OSCC to the mandible of unknown origin 3(1.9%) and these were excluded in the analysis. Majority of the well differentiated OSCC was in the tongue 31(57.4%) followed by the buccal mucosa 18(24.3%). Of the poorly differentiated OSCC a majority were also in the tongue 18(27.7%) followed by the hard palate 13(20.0%) and maxilla 11 (16.9%. The distribution of histological differentiation by tumour site is as shown in table 3 below.

SITE	WELL	MODERATELY	POORLY
TONGUE	31(41.9%	4(7.4%)	18(27.7%)
MANDIBLE	7(9.5%)	2(14.3%)	4(6.2%)
MAXILLA	4(5.4%)	1(6.2%)	11(16.9%)
LIP	7(9.5%)	1(7.2%)	5(7.7%)
HARD PALATE	6(8.1%)	1(5.0%)	13(20.0%)
SOFT PALATE	0(0.0%)	0(0.0%)	1(1.5%)
BUCCAL MUCOSA	18(24.3%)	2(6.7%)	10(15.4%)
GINGIVA	1(1.4%)	0(0.0%)	2(3.1)
OROPHARYNX	0(0.0%)	0(0.0%)	1(1.5%)
TOTAL	**74(48.4%)**	**11(7.0%)**	**65(42.7%)**

Table 3 Histological differentiation by tumor site

A Spearman correlation coefficient test was used to determine the association between tumor sites and histological differentiation and it elicited a weak association which was non-statistically significant (rs =.114 and p=.184).

3.4 TP53 gene analysis

This study carried out DNA extraction and analysis on a random sample of 20 paraffin-embedded samples. Complete DNA sequencing was done on 19/20 of the samples. Sequencing was done on exons 6 and 7 both forward (5' to 3') and backwards (3' to 5') giving a total of 38 samples sequenced. The sequencing pattern was then assembled using the Sequencher® software while alignment of the nucleotides was done using both the UGene® and the Mega® softwares for DNA sequencing. An example of the results as seen on the Mega® software before alignment is shown in figure 8 below.

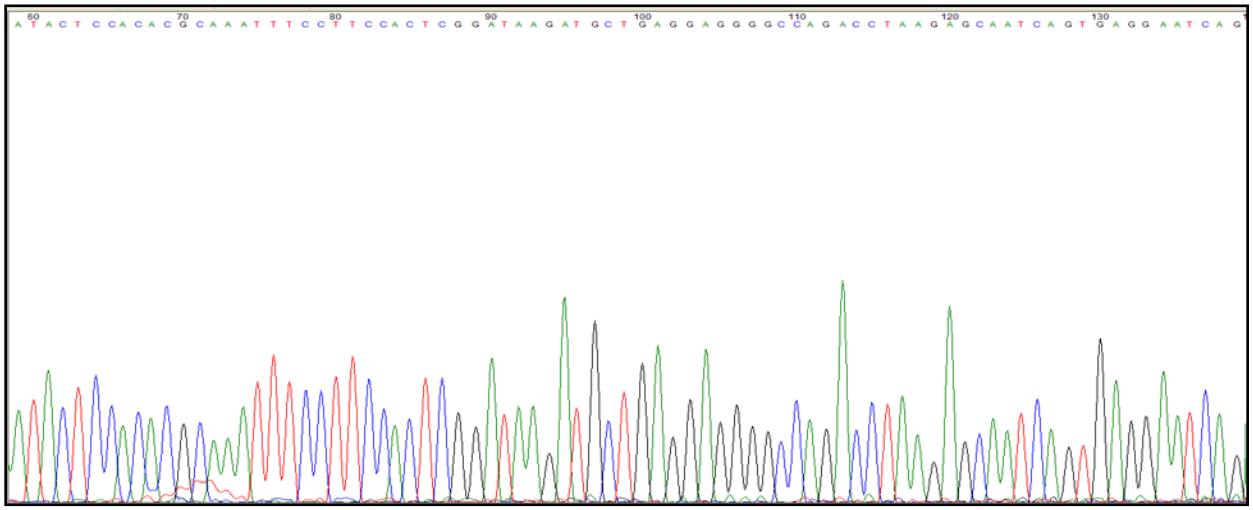

Figure 8 Sample results of DNA sequencing

The evenly-spaced peaks each with only one colour represent good quality of nucleotides sequenced and ready for alignment and analysis. The baseline peaks ("noise") appearing at the bottom are also minimal.

The nucleotide sequences were then compared with the normal human p53 gene sequence to check for mutations. The DNA sequence for normal human TP53 (more than 19,000 nucleotides) is available in the humane genome database of the IARC. An example of the results after alignment is as shown in figures 9 below. No mutations were found in the exons.

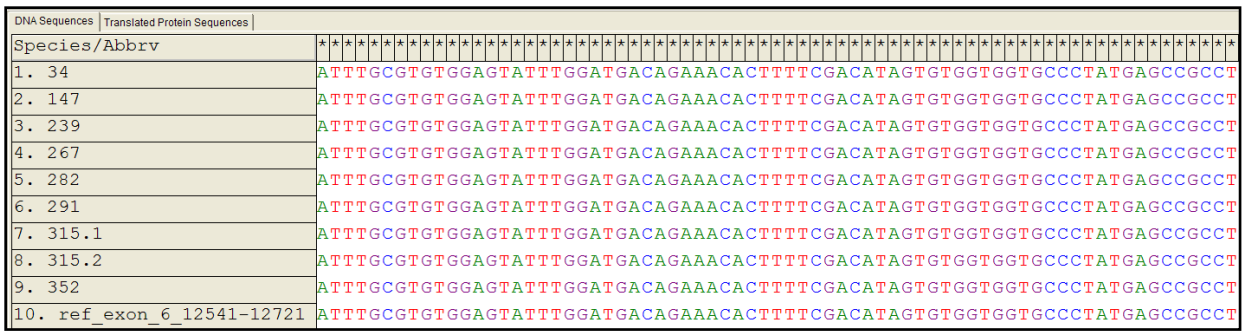

Figure 9 Sequencing aligned against normal human TP53 (labelled 10 in the figure) showing no mutations.

This shows 9/20 samples as well as the normal human p53 reference. Matching nucleotides indicate that no mutation is present.

The study found 6 single nucleotide polymorphism (SNPs) in 5 tumour samples. Table 4 below summarises the characteristics of the 5 samples.

Age	Gender	Hospital	Site	Differentiation	SNPs Type	SNPs Location
74	M	MACHAKOS	MAXILLA	MODERATELY WELL	C-A	INTRONIC
18	M	MERU	MAXILLA	POORLY	C-A	INTRONIC
35	M	MERU	TONGUE	WELL	C-A	INTRONIC
64	M	SDS	MANDIBLE	POORLY	C-A	INTRONIC
70	F	MACHAKOS	BUCCAL	WELL	C-A & T-G	INTRONIC

Table 4 Characteristics of the samples in which SNPs were found

The mutations found were all of the SNPs type and were located in the introns. Out of the 5 samples with SNPs 4 (80%) were male and 1(20%) was female. The median age of these samples was 52.2 years. The maxilla was the tumour site of SNPs in 2/6 samples representing 33.33%. The SNP resulting in C-A substitution was the commonest at 5/6 (83.3%). The SNPs resulted in coding for a different protein; the C-A substitution coding for Glutamate (Q) instead of Histidine (H) while the T-G substitution resulted in coding for Cysteine (C) instead of (G).

4.0 CHAPTER 4 – DISCUSSION CONCLUSION AND RECOMMENDATIONS

4.1 DISCUSSION

This study focused on four aspects of oral squamous cell carcinoma (OSCC). Firstly, we studied the socio-demographic characteristics of the OSCC patients diagnosed at UoNDH. Secondly, the clinical aspects of oral cancer including presenting complaints, tumor site, symptom duration, and treatment modalities offered to patients in Kenya were described. Thirdly, the histological patterns of OSCC were also studied. Finally, we analysed a representative sample of 20 FFPE tissue blocks for mutations in TP53 gene. Since this was a pilot study it was not considered appropriate to analyse TP53 mutations in all the 157 patients from whom the diagnosis of OSCC had been made during the study period. However, the patient characteristics in all 157 patients were analysed to establish whether or not there were significant differences among these patients.

4.1.1 Socio-demographics

This study has shown that the gender distribution for OSCC is 1:1 and this is consistent with a previous study in Kenya which reported a slight male predominance at 1.3:1[3]. However a recent study in a Kenyan population reported a male: female ratio of 1.6:1[25]. The male predominance for OSCC may be attributed to the fact that the aetiological factors like smoking and alcohol use are more common in males. Tobacco use is reported at 15.1% among males and 0.8% among females in Kenya[26]. The finding of a gender ratio of 1:1 in the present study could be because the females also tend to be exposed to secondary smoke from the males smoking in their households. The male to female ratio has been shown to vary depending on geographic region. A South African study reported a male to female ratio of

2.9:2.1 [27]. In high prevalence regions like South East Asia the reported prevalence is the same in both genders[28] which is similar to this study.

The mean age (57 ±15.92 SD years) at presentation is similar to other studies which have found OSCC to occur more in the 5th and 6th decades of life [3, 29]. However Muange et al. (2014)[25] reported a peak age occurring a decade later. Our study, however, found a second peak in incidence of oral cancer in the age group 61-75 years. Notably in our study, 22.3% of patients were younger than 44 years which differs from two other studies in Kenya; Muange et al. (2014)[25] who reported 13.4% of patients aged below 40 years and Dimba et al (2007) [30] who reported 10.3%. A similar study in South Africa reported only 9% of patients younger than 40 years[27]. It is likely that we have OSCC occurring increasingly in the younger age groups because of earlier use of tobacco products and alcohol. The role of HPV in the aetiology of OSCC is also now well established and could explain the increasing number of young adults with OSCC. It is projected that the new cases of HPV related OSCC will surpass those of cervical cancer by the year 2030[2].

This study found that the females were older than the males and this was statistically significant. The males likely get OSCC earlier because of the use of tobacco and alcohol from an early age.

A majority of patients came from the Meru region and tribe. This part of Kenya is known for khat growing and has a high percentage of khat users and this may be a contributing factor to the high numbers of OSCC from that region. The patients from Meru were also relatively younger (mean age 53.25 years) than those from other parts of Kenya, though this was not statistically significant. Notably the youngest patient, age 11 years, was from Meru. The high incidence of OSCC in the Meru region coupled with occurrence in younger age groups may be an indication of yet unidentified carcinogenic factors that are possibly peculiar to this geographic population.

4.1.2 Clinical characteristics

The most common complaint at presentation was an ulcer in 107/157 cases (75.9%) followed by a swelling 24/157 (17.0%) and this is similar to an earlier Kenyan study which reported an ulcer as the commonest symptom at 97.6% followed by pain 93.9% [25]. We did find that the symptom duration varied widely from less than 2 weeks to over 1 year. Muange et al. (2009) [25] also reported a mean duration of symptoms at presentation to be 7.2 months. In our study most patients (51.5%) however, presented within 6 months of noticing their symptoms. Despite this seemingly early presentation, most patients had advanced disease at the time of diagnosis in our referral center. It appears that patients take a very long time from the initial onset of symptoms to the time they have a diagnosis. This could be attributed to scarcity of resources and unavailability of specialised oral cancer diagnosis facilities in peripheral facilities.

The commonest tumour site for OSCC was the tongue at 54/157 (35.3%) and this has been reported in other studies as well [3, 25, 30]. It is also not uncommon to find advanced tongue cancer extending to involve the floor of the mouth and thus being listed as the latter. The lip (both upper and lower) appears to be uncommon in our patients. Only 1 patient had OSCC involving the upper lip. We also found that occasionally clinicians do not indicate the site of tumour in the histopathology request forms despite this being part of the standard request form.

The staging of OSCC using the TNM classification is considered a gold standard in the management of these patients. However, TNM staging was not done in over 80% (126/157) of the cases we studied over the two year period. This despite the emphasis placed on TNM staging and its significance in planning management of OSCC. This study found that the TNM stage is not routinely indicated both in the

patient notes and in the histopathology request forms. This is possibly because biopsy procedures are not routinely done by the oral and maxillofacial surgeons who would be expected to be more interested in the TNM staging of these tumours. The histopathology request forms also do not provide for the TNM stage to be indicated. However even for the patients offered treatment after a definitive diagnosis the staging of OSCC was not done routinely. Where TNM staging was done (31/157, 19.7%), a majority of patients were in Stage III and IV (30/ 31 or 96.8%). This confirms as in other local studies[3, 25] that a majority of our patients receive their definitive diagnosis and treatment plans when they have advanced disease. Notably, there was no patient in this study who presented in Stage I OSCC. This is as a result of the long duration of time that most of these patients waited from onset of symptoms to the making of a definitive diagnosis.

This study found that a majority of patients are either lost to follow up after the initial diagnosis or no record of treatment modalities was provided. This is probably because of the very advanced disease at presentation which more often requires palliative care. It is also possible that these patients sought treatment elsewhere once a diagnosis was made. However we could not establish in the present study the status of these patients who were essentially lost to follow up. We did however find that for those in whom some form of treatment was provided, the mean duration from diagnosis to treatment was 6 months. This is a long waiting period for a patient with OSCC and likely contributes to poor outcomes.

4.1.3 Histological characteristics

In this study, a majority of OSCC were well differentiated, constituting 48.4% of the tumours. This is similar to other studies reported in literature which have found most OSCC to be well differentiated followed by poorly differentiated [29, 31, 32].However a recent study by Muange et al. (2009)[25] reported that

most OSCC were poorly differentiated at 48.8%. A South African study reported that the moderately well differentiated variant was the commonest (80%) followed by poorly differentiated (14%)[27].

In our study, OSCC of the tongue was more likely well differentiated while tumours of the maxilla and the buccal mucosa were mainly poorly differentiated. The significance of this in relation to prognosis was beyond the scope of the present study. It has been shown that the histological variant reflects the aggressiveness of OSCC though multivariate analyses have not shown this to be an independent prognostic factor [33]. In some centers the histological variant is used in determining the use of neoadjuvant and/or adjuvant radiotherapy in treatment protocols[34].

4.1.4 TP53 mutations and polymorphism

The reported prevalence of p53 mutations in head and neck cancers varies from 16%[16] to as high as 93%[17]. These wide variation in reported mutations has been widely attributed to heterogenicity of head and neck tumours, differences in methods of gene analysis, varied sample sizes in different studies, and even geographical variations [12]. To our knowledge this is the first study in Kenya and the region that has attempted to study the pattern of p53 gene alterations in OSCC. We analysed DNA from paraffin-embedded tissue blocks and found no gene mutations in exons 6 and 7; prevalence 0%. This varies from other studies in literature which have reported p53 gene mutations in exons 6 and 7 as well as in other exons [7, 12, 17]. However the fact that we only studied exons 6 and 7 could explain this finding. Studies that analysed more exons have tended to report higher prevalence of p53 mutations. It is our postulate that had we studied exons 5-8, or even the entire exons 2-11 that make up the p53 gene, the prevalence of p53 gene mutation would have been higher and more consistent with published literature. However, we found 6 single-nucleotide polymorphisms (SNPs) in 5/20 samples that were analysed. All 6 SNPs

were in the DNA binding domain (DBD). Indeed, the only SNP reported previously in the p53 DBD is on codon 217 (Valine for Methionine)[35]. To our knowledge this is the second finding of polymorphisms in the DBD. Most of the reported TP53 polymorphisms have been located in the introns and this is consistent with our study in which all the 6 polymorphisms were located in the introns[13, 14, 22].

The most studied SNP in TP53 is the Proline/Arginine variation at codon 72 which is due to a change in the DNA sequence encoding the proline-rich domain of p53 (CCC to CGC)[14]. Commonly reported SNPs in literature include Proline to Serine at codon 47 resulting from a C to T substitution which has been found in populations of African origin [36]. The polymorphisms found in our study were however, C to A and T to G substitutions. Additional SNPs that appear specific to Chinese populations were recently reported, but there impact on cancer risk remains to be evaluated in large cohorts [24]. Whether the SNPs reported in our study are specific to a Kenyan/African population needs to be confirmed in larger genome studies.

Tumour-associated mutations in TP53 in the coding sequence are a hallmark of most human cancers and have been shown to cause dramatic effects in p53 function. The functional consequences of the more than 200 naturally occurring sequence variations (SNPs) of the TP53 in human populations are theoretically unknown [15]. SNPs are thought to affect p53 protein function through enhanced mutation potential[37].

Significant ethnic differences in codon 72 polymorphism have been observed. The Pro72 allele is more common in Western Europe and in Africa (Nigerians), while in USA, Central and South America and Japan , the most common allele is Arg72 [22, 38]. Some authors have hypothesised this geographical

variation to be due to winter temperatures and UV light but this hypothesis has not been proven [38]. The SNPs found in our study have not been previously reported and it remains to be established whether they are specific to this geographical region.

Whether the SNPs found in our study have an effect on the p53 function was beyond the scope of our current research. Studies done elsewhere on SNPs have demonstrated their impact on p53 behaviour. The Proline/Arginine polymorphism at codon 72, for example, was reported to be more efficient in inducing apoptosis than does the normal Proline[13].

This study has revealed single nucleotide polymorphisms (SNPs) in the DNA binding domain (DBD). This finding has only been reported once previously [35]. In addition, the SNPs we found in our study at codon 220 (Glutamate/Histidine) and codon 254 (Cysteine/Glycine) have never been reported, the significance of which is yet to be established.

4.2 Conclusion

This study found that there is a male to female ratio of 1:1 for OSCC and that most patients were in the 5th to 6th decade. The commonest tumour site was the tongue with an ulcer being the commonest presenting symptom. A significant number of tumours in all sites are histologically poorly differentiated. A majority of OSCC in this study were from the Meru ethnic community.

The study found 6 intronic single nucleotide polymorphisms (SNPs) in 5/20 samples of confirmed oral squamous cell carcinoma (OSCC) in a Kenyan population. This finding is consistent with other published studies which have reported intronic SNPs. In addition, the SNPs in our study were all located in the DNA binding domain (DBD). Since we only studied exons 6 and 7, the 0% prevalence of TP53

mutations we found in this study is not representative for TP53 gene mutations in our population. Neither is the finding of SNPs in 5/20 samples that were analysed. Our sample size at present is too small to make such a conclusion. There are possibly other unreported SNPs in our population especially considering that this study focused on exons 6 and 7.

This being a pilot study does however, open room for more comprehensive studies in future to attempt to answer the question “what is the prevalence and pattern of TP53 gene mutations and polymorphisms among OSCC patients in a Kenyan population?”

4.3 Recommendations

- There is need for programs to create awareness on symptoms of oral cancer among the general population
- There should be training of health care workers on early detection of oral cancer.
- Further epidemiological studies are required on patterns of OSCC in Kenya with the aim of establishing whether there are any peculiar aetiological factors in some geographic regions.
- Further studies should be done on the prevalence and pattern of TP53 mutations and polymorphisms in our population.
- That a protocol is put in place to guide future research in the field of head and neck cancer molecular biology and genetics in Kenya.
- Collaborations between clinicians who treat head and neck cancer patients and scientists who study cancer genetics in this institution should be encouraged, nurtured and supported.

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