SERUM ANALYSIS OF ALBUMIN AND ANTIOXIDANT VITAMINS AND SOCIO-DEMOGRAPHIC STUDIES IN ORAL CANCER PATIENTS IN IBADAN

BY

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MAY 2008
ATTESTATION BY SUPERVISORS

This is to certify that we supervised this project undertaken by Dr. A.O. Lawal entitled Serum Analysis of Serum Albumin and Antioxidant Vitamins and Socio-Demographic Studies in Oral Cancer Patients in Ibadan.

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DECLARATION

I declare that this work was done by me, Dr Lawal Ahmed Oluwatoyin under appropriate supervision by my consultants. This dissertation has never been presented to any examining body nor has it been submitted elsewhere for publication. The findings and opinions in this work are entirely mine and should not be taken as representing the views of the University College Hospital, Ibadan.

Dr. A.O. Lawal
DEDICATION

This work is dedicated to my sweet heart, my friend and my wife, Toyosi for her Love and support and to my son John, who is such a wonderful gift from God.
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SUMMARY

This case control study compared serum albumin and antioxidant vitamin levels in oral epithelial cancer patients and a control population that presented at University College Hospital (Dental Centre), Ibadan and Adeoyo State Hospital, Ibadan. Sixty-two patients were enrolled for the study, comprising 32 clinically diagnosed oral carcinoma patients and 30 control patients. Fasting blood samples of all participating patients were centrifuged at 3,000 rpm for 5 minutes and separated serum was analysed for albumin, vitamins A, C and E, using a DM520 spectrophotometer. The oral cancer group had a biopsy done to determine histological diagnosis. A 33-item self administered questionnaire containing sections on bio-data, social habits (including nutrition) and knowledge of oral cancer, was administered to all enrolled patients. The data obtained was analyzed using the SPSS Statistical Package (SPSS, Version 11.0). The results of the study showed that oral cancer occurred more frequently in females, with a male to female ratio of 1:1.7. The mean age for oral cancer patients was 53.7 years. 81.3% of the oral cancer cases occurred above 40 years of age. The mean serum micronutrient values obtained were; serum albumin (3.5 ± 0.7g/dl), vitamin A (0.53 ± 0.26mg/L), vitamin C (0.63 ± 0.28mg/dl) and vitamin E (6.68 ± 3.03mg/L) in oral cancer patients. These were significantly lower than those of the control group, who had mean values of (4.5 ± 0.49g/dl), (0.81 ± 0.28mg/L), (0.91
± 0.25 mg/dl) and (8.80 ± 3.49 mg/L) for serum albumin, vitamin A, vitamin C and vitamin E respectively with (p = .000), (p = 0.22), (p = .000) and (p = .013) respectively. The average income of the oral cancer patients was significantly lower than that of the control group (p = 0.018). Although there was an inverse relationship between educational status and occurrence of oral cancer, there was no statistically significant difference between the educational status of oral cancer patients and the control patients (p = 0.22).

In this study, oral cancer occurred more in individuals older than 40 years. Furthermore, there was a significant difference between the income of oral cancer patients and the control group. Also observed in this study, was a statistically significant difference between the mean serum levels of albumin, vitamin A, C, and E in oral cancer patients when compared with control patients. More studies examining the relationship between nutrition and oral cancer need to be carried out, especially in West African sub-region. Nutritional and dietary counselling with regard to the importance of fruits and vegetables consumption need to be re-emphasised in community oral health education programmes. Furthermore, similar case control studies using larger sample size are recommended.
CHAPTER ONE

INTRODUCTION

Oral cancer is defined as a malignant neoplasm involving the oral cavity, which is a region extending from the lips to the anterior pillars of the fauces. Oral cancers rank amongst the ten most common cancers worldwide and show marked geographical variation in occurrence. The highest rates of oral cancer have been reported in developing countries, particularly India, Sri Lanka, South Vietnam, Papua New Guinea, the Philippines, Hong Kong and Taiwan.

Oral malignant neoplasms represent 2-4% of all malignant lesions in the United States of America, 2% of cancers in Britain and 1% in Australia. Oral cancers are the commonest cancers amongst men in India and number three amongst women, accounting for 15 to 50% of all
cancer cases in India. This may be due to high prevalence of social habits such as tobacco and betel nut chewing in India.

The trend in survival rate of patients with oral cancer has been rather disappointing with overall 5 years survival rate of about 50%, without much improvement over the decades.

Many factors have been implicated in the aetiology of oral cancers. Among these, tobacco is regarded by most authors as the most important aetiological factor. All forms of tobacco smoking are linked to oral cancer, with cigar and pipe smoking said to be associated with greater risk of oral cancer. Other important aetiological factors include alcohol, radiation, immunosuppression and nutrition.

However, some authors have reported oral cancer in people who do not appear to smoke or consume appreciable amounts of alcohol. This supposes that there is the possibility of other important factors in the aetiology of oral cancer. Conspicuous national and international variations in oral cancer incidence and mortality rates as well as observations of altered incidence rates in migrant populations have raised the possibility that diet and nutritional status may be an important aetiological factor.

Early case control studies examining the role of diet in the aetiology of oral cancer found little difference in nutritional status of oral cancer patients compared with normal population. However, long
standing iron and other nutritional deficiencies resulting in Plummer Vinson syndrome have been shown to predispose to oral and pharyngeal cancers\textsuperscript{15}. Some authors have shown that diets high in fruits and vegetable, and vitamins A and C \textsuperscript{16, 17} may protect against oral cancer while others reported no association between oral cancer and vegetable intake. \textsuperscript{18}

Some studies have shown that oral cancer patients have lower serum antioxidant vitamin levels when compared with those of normal cancer free patients \textsuperscript{19,20,21,22}. Kune \textit{et al} in a study in Australia showed that mean serum β-carotene and vitamin A were significantly lower in oral cancer patients than in controls \textsuperscript{19}. Ramaswamy in India also found a significantly lower serum level of Vitamins A and C in oral leukoplakia patients compared to a control population \textsuperscript{20}. Other studies in USA \textsuperscript{21} and Japan \textsuperscript{22} also found lower serum antioxidant vitamins in oral cancer patients than in normal population.

Oji \textit{et al} in Enugu had previously reported that most of the oral cancer patients presenting at University of Nigeria teaching Hospital (UNTH) over a six year period, gave a negative history of alcohol and tobacco misuse. They suggested that the cycle of poverty, malnutrition, lack of education, poor oral hygiene and chronic malaria may play an important role in aetiology and severity of oral cancer\textsuperscript{23}. Their finding is in agreement with Lawoyin \textit{et al} who in their study in south-western
Nigeria, also found low prevalence of recognised risk factors such as tobacco and alcohol consumption for oral cancer in their patients and suggested other predisposing factors such as nutrition, genetic predisposition and the role of chronic illness.

There is a need to contribute to general knowledge of oral cancer more especially the role of nutritional factors in the aetiology of oral cancer in Nigeria, where there is a paucity of studies done is this respect. This study will therefore analyse the serum albumin and antioxidant vitamins in oral cancer patients and compare them with those of controls in order to validate the probable role of micronutrient deficiencies in the aetiology of oral cancer in a Nigerian population.
CHAPTER TWO

AIM AND OBJECTIVES

2.1 AIM

To determine the socio-demographic factors and the serum levels of albumin and antioxidant vitamins in oral carcinoma patients and compare them with those of control patients attending the dental outpatient clinics of the University College Hospital (UCH) and Adeoyo State Hospital, Ibadan.

2.2 OBJECTIVES

- To determine the socio-demographic data of patients with oral cancers as compared with control cases at UCH and Adeoyo State Hospital Ibadan.
- To determine serum levels of albumin and antioxidant vitamins in oral cancer and control cases.
- To determine the relationship between the socio-demographic data and serum levels of albumin and antioxidant vitamins in all enrolled patients.
- To compare the prevalence of habits such as tobacco use, alcohol consumption and fruits and vegetable consumption among oral cancer and control cases.
CHAPTER THREE

LITERATURE REVIEW

3.1 Epidemiology of Oral Cancer

Oral cancer is defined as a malignant neoplasm involving the oral cavity, which is a region extending from the lips to the anterior pillars of the fauces\(^2\). Oral cancer is estimated to be the sixth most common cancer worldwide, the most common histological type being squamous cell carcinoma\(^{25}\).

There is a great geographic variation in the incidence of oral cancer worldwide with highest prevalence rates of 35 to 50% occurring in India\(^{25}\).

In 1988, about 30,000 new cases of oral cancer were diagnosed in the United States\(^{26}\). Oral cancer is relatively rare in the United Kingdom, with about 2000 new cases diagnosed each year. An increase in incidence has been reported in central and eastern Europe especially among men\(^{27,28}\).

Oral cancer is believed to be relatively rare amongst Africans\(^{29}\). Arotiba et al., in a review of 246 oral squamous cell carcinoma patients in University College Hospital Ibadan, reported 1.2% of all malignant lesions to be oral squamous cell carcinoma\(^{30}\). This was, however, higher than the 0.4% earlier reported by Abiose et al from the same centre who examined oral soft tissue malignancies\(^{31}\). Oji in Eastern Nigeria reported that oral cancer accounted for 2.7% of all cancer cases seen at the University of Nigeria Teaching Hospital (UNTH) Enugu over a six years
period\textsuperscript{23} while Otoh \textit{et al} in Maiduguri, North-eastern Nigeria, reported an average rate of 20 cases per annum over a six years period\textsuperscript{32}.

\textbf{3.1.1 AGE DISTRIBUTION}

The incidence of oral cancer increases with age, although the pattern differs markedly in different countries with different associated risk factors. In the west, 98 percent of cases were over 40 years of age and the incidence rises from an overall average of 3-4 cases per 100,000 per annum at all ages, to 100 cases per 100,000 per annum in those over 75 years of age\textsuperscript{33}. The increased incidence of cancer with advancing age may be partly due to the increasing level of free radical reactions with age. Also, there is said to be a diminishing ability of the immune system to eliminate altered cells and the effectiveness of cancer surveillance by immune cells is reduced over the years\textsuperscript{34}.

However, in regions with high rates, many cases occur before the age of 35 years particularly in heavy abusers of tobacco and also teenagers who are involved in the use of smokeless tobacco\textsuperscript{35}. This habitual pattern of heavy tobacco use also accounts for oral cancer cases occurring in third and fourth decades in western countries.\textsuperscript{35,36}

In a study in Zimbabwe, Chidzonga reported a peak age incidence in the sixth decade accounting for 35\% of oral squamous cell carcinoma seen\textsuperscript{37}. Arotiba in Ibadan, Otoh in Maiduguri and Odukoya in Lagos all reported
peak age incidence in the sixth decade\textsuperscript{30, 32, 38}, while Oji in Enugu reported peak incidence in the seventh decade.\textsuperscript{32}

3.1.2 SEX DISTRIBUTION

Oral cancer affects males about twice as often as females in the industrialized world, possibly due to a greater exposure of males to established risk factors like tobacco and alcohol use. This difference is not observed in countries such as India where exposure to risk factors is almost equal in both gender. Also, no difference was noted in incidence of oral cancer between men and women in some ethnic groups in Singapore, Hawaii and in Denmark where exposure to risk factors such as tobacco use and alcohol consumption are similar between males and females.\textsuperscript{33} Arotiba et al reported a male to female ratio of 1.5:1 in southwest Nigerian population\textsuperscript{30}. Odukoya et al and Ajayi et al in Lagos reported a male to female ratio of 1.3:1 and 2:1 respectively.\textsuperscript{38, 39} Oji in Enugu also reported a male to female ratio of 1.5:1\textsuperscript{23} but Otoh in Maiduguri, found a male to female ratio of 3:4\textsuperscript{32} and suggested that the higher preponderance of oral cancer in females in their study, may be attributed to the increasing exposure of females in North-eastern Nigeria to carcinogens such as tobacco and alcohol.
3.1.3 ETHNIC INFLUENCES

Ethnic variation influences prevalence, largely because of social and cultural practices. Where these represent risk factors, their carriage by emigrants from high-risk areas to other parts of the world, results in comparatively higher cancer incidence in immigrant communities\textsuperscript{40}. In the UK, oral cancer was found to be more prevalent in areas with high Asian population; this was thought to be due to high prevalence of tobacco chewing among the Asian population\textsuperscript{41}.

In Australia, migrants from the Mediterranean and Middle East have lower rates of mouth cancer than the Australian-born population and this was attributed to the higher prevalence of habits such as tobacco use and alcohol consumption in the Australian-born population\textsuperscript{41}.

3.1.4 SITE DISTRIBUTION

The lip is the most common site of oral cancer in fair skinned races particularly in men who work out of doors due to their exposure to ultraviolet radiation from the sun\textsuperscript{40}. Intraoral cancer in western countries most commonly affects the lateral border of tongue and the floor of the mouth; this is probably due to the effect of pooling of saliva on the floor of mouth and tongue by gravity. Saliva acts as solvent for carcinogens in alcohol and tobacco and prolongs the contact time of these carcinogens with tongue and floor of mouth. The buccal mucosa is the next most common site of occurrence followed by mandibular alveolus, retromolar
region and soft palate with the hard palate and dorsum of tongue having the lowest risk sites. In the high incidence areas of south East Asia, the buccal, retromolar, and commissural mucosa are the most prone sites\textsuperscript{33}. Nigerian studies carried out in Ibadan population however show that the tongue, palate and the mandibular alveolus are the sites most commonly affected with the floor of mouth and buccal mucosa being the least affected\textsuperscript{30,42}. Odukoya \textit{et al} in Lagos reported that mandibular gingiva, maxillary gingiva, hard palate, floor of mouth and tongue were the commonest sites of occurrence, while buccal mucosa, upper lip, lower lip and oropharynx were the least affected sites in their study\textsuperscript{38}.

\textbf{3.1.5 TIME TRENDS}

In the urban regions of countries with high incidence of oral cancer such as Bombay, a reduction in the incidence of oral cancer and a concomitant rise in lung cancer has been noted. This has been attributed to a change of tobacco habits with a move from local agents such as bidi to smoking of manufactured cigarettes. The incidence however remains high in rural India\textsuperscript{33}. A rising trend in tongue cancer incidence, particularly among younger men, has been shown for many western countries including USA\textsuperscript{35}, Scotland\textsuperscript{43} and Nordic\textsuperscript{44} countries where occurrence of oral cancer has been attributed to increase use of alcohol and tobacco in young people.
3.1.6 MORTALITY

Intra-oral cancer has a high mortality rate but that of the lip is much less. The crude 5-year survival rates are 80% for lip and 30-40% to for intra-oral cancers\textsuperscript{33}. The trends in survival of patients with these oral cancers have been rather disappointing over several decades. The overall survival rates of all patients with oral cancer are about 50%. The survival rate for black Americans has been estimated to be significantly lower than for white Americans\textsuperscript{9,10} with 5 years survival rate of 33% in black Americans\textsuperscript{41}. The poorer survival rates in blacks have been attributed to their lower social-economic status, poorer overall nutrition, more advanced stage of presentation and differences in the type of treatment they received due to poor access to appropriate health care\textsuperscript{41}.

Within the mouth, factors that influence survival are site (generally, the further back in the mouth the location of the lesion, the worse the prognosis, because of poor accessibility for surgery); the size of the lesion at diagnosis (reflecting the clinical stage); its degree of differentiation (poorly differentiated tumours have an adverse prognosis), the involvement of regional lymph nodes (reflecting the clinical stage), and whether or not blood borne metastases are present (reflecting the clinical stage)\textsuperscript{33}. 

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3.2 AETIOLOGY
3.2.1 TOBACCO
The use of tobacco in whatever form is associated with increased risk of intra oral cancer worldwide\(^\text{45}\). It is commonly consumed in betel quid or pan consisting of tobacco mixed with chopped areca nut, slaked lime and catechu, wrapped in a leaf of piper betel vine. In Indians, spices such as cardamom, cloves and aniseed may be added\(^\text{33}\).
In North Africa and the Middle East, a mixture of tobacco, ash and lime in water or oil called nass or nasswar, is commonly held in the mouth. Many different forms of snuff are placed in contact with oral mucosa in northern Europe, France, the USA and parts of Africa including Sudan, South Egypt and Saudi Arabia\(^\text{33}\). Users of tobacco quid especially if they also smoke, have 10 to 20 time’s greater risk of developing oral cancer than those who neither chew nor smoke\(^\text{34, 35}\).
All forms of tobacco use have been strongly linked to oral cancer. Cigar and pipe smoking are linked to a greater risk of the development of oral cancer than with cigarette smoking probably because most cigarettes have filters that reduce the load of carcinogen that will come in contact with the mucosa\(^\text{9}\). Reverse smoking (the habit of holding the lighted end of the cigarette inside the mouth) as may be practiced in India and some South American countries, is associated with a significantly higher risk of oral cancer due to high intensity of tobacco combustion in close proximity to the oral mucosa.\(^\text{9}\) Nitrosamines an important constituent of tobacco is a
known carcinogen \textsuperscript{48, 49}. Carcinogens in tobacco (mainly polycyclic aromatic hydrocarbons), can cause an accumulation of genetic mutations in oral epithelial cells including \textit{P53} mutation, mutation, loss of heterozygosity (H-\textit{RAS}) and amplification (K-\textit{RAS} and N-\textit{RAS}) of the \textit{RAS} oncogenes leading to abnormal and uncontrollable cell division and growth.\textsuperscript{41}

\textbf{3.2.2 ARECA NUT}

It is unclear whether chewing areca nut without added tobacco increases the risk of developing oral cancer\textsuperscript{45}. Although the habit is associated with the development of oral sub mucous fibrosis\textsuperscript{24} which itself is associated with a greater than 7 per cent risk of progressing to malignancy over a number of years\textsuperscript{50}.

\textbf{3.2.3 ALCOHOL}

Alcohol was formerly not believed to be a carcinogen but appears to add to the risk of oral cancer development\textsuperscript{9}. Identification of alcohol alone as a carcinogenic factor has proved to be somewhat difficult because of the combination of smoking and drinking habits by most patients with oral cancer. The effect of alcohol has been thought to occur through its ability to irritate the oral mucosa and to act as a solvent for carcinogens (especially in tobacco).\textsuperscript{9}Contaminants and additives with carcinogenic potentials that are found in alcoholic drinks have also been thought to have a role in oral cancer development. Molecular studies have suggested
that carcinogenic risks associated with alcohol may be related to the effect of an alcohol metabolite, acetaldehyde formed by the action of the enzyme alcohol dehydrogenase. Acetaldehyde causes the alteration of keratinocyte genes such as \textit{P53} tumour suppressor gene and \textit{RAS} oncogenes\textsuperscript{9}.

\textbf{3.2.4 FUNGAL INFECTIONS}

Hyphae of \textit{Candida albicans} are frequently seen invading the outer epithelial layer in oral white and red patches with known malignant potentials, and sometimes in oral cancer itself\textsuperscript{51}. \textit{Candida albicans} has been suggested to be a possible causative agent of oral cancer because of its potential to produce a carcinogen, N-nitrosobenzylmethylamine\textsuperscript{9}. This is due to the catalytic ability of \textit{Candida albicans} to produce this carcinogen from precursors N-benzyl methylamine and nitrites by a process of nitrosation\textsuperscript{52}.

\textbf{3.2.5 VIRUSES}

Studies have demonstrated the occasional presence of human papilloma virus (HPV) subtypes 16 and 18 in oral squamous cell carcinomas, suggesting a possible role for this virus in oral cancers. This association is strongest for oropharyngeal squamous cell carcinomas where up to 50\% of tumours from this site may contain evidence of HPV\textsuperscript{9}. 

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3.2.6 ULTRAVIOLET RADIATION

This is a major carcinogen for skin cancer (basal cell carcinoma, squamous cell carcinoma and malignant melanoma) on the face and other exposed areas, particularly in fair skinned individuals. It is also important in the aetiology of squamous cell carcinomas of the vermilion border of the lip, particularly in those who live or work outdoors\textsuperscript{40}.

3.2.7 IMMUNOSUPPRESSION

A compromised immune system puts patients at risk for developing cancers including oral cancer. This increased risk has been documented for bone marrow and kidney transplant recipients, who are subjected to iatrogenic immunosuppression. The total body radiation and high dose chemotherapy that are used to condition patients for bone marrow transplants also put patients at a life long risk for solid and lymphoid tumours\textsuperscript{9}. This increased risk is attributed to the reduced ability of the immuno-compromised patient to mount immuno-surveillance to cancer cells that are formed even in normal patients\textsuperscript{34}.

HEREDITARY FACTORS

Although habits such as tobacco and alcohol consumption are thought to be the most important factors involved in the aetiopathogenesis of oral cancer, some authors have suggested a role for hereditary/ genetic factors. The molecular changes found to be associated with oral carcinomas in western countries (UK, USA and Australia) are mainly $P53$ mutations but
these are rare in the east (India and south East Asia) where more of RAS oncogenes abnormalities are common. This suggests that there may be differences in genetic injuries that lead to oral cancer between populations from these different geographic areas\textsuperscript{41}.

Some researchers have suggested that there could be genetic differences in the ability to metabolise pro-carcinogens and carcinogens by means of xeno-metabolising enzymes or in the ability to repair DNA damage between different ethnic groups. Alcohol dehydrogenase (ADH) type 3 genotypes appear to be more prone to oral cancer because they have high activity of ADH which metabolises ethanol to acetaldehyde. Acetaldehyde is known to be cytotoxic and results in free radicals production and DNA hydroxylated bases \textsuperscript{54}.

Glutathione S- transferase (GST) detoxifies hydrocarbons epoxides. The genotypes GSTM1 and GSTP1 are able to metabolise benzpyrene and other polycyclic aromatic hydrocarbons (PAH). Some genotypes are said to have impaired activity; for example null genotypes of GSTM1 and GSTP1 have decreased ability to detoxify tobacco carcinogens and have been shown to predispose to oral cancer in some studies \textsuperscript{55}. Patients with oral cancer have been shown to have increased sensitivity to various mutagens and show evidence of defective DNA repair, traits that correlate with positive family history of oral cancer\textsuperscript{56}. 

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3.2.8 DIET AND NUTRITION

Nutritional deficiency produces atrophy of oral and other mucous membranes and may render them more susceptible to local carcinogens. Diets high in vitamin A and C, derived from high fruit and vegetables, appear to confer some protection against cancer. In a study in Kerala, India, oral administration of β-carotene and vitamin A produced remission of oral leukoplakia and nuclear abnormalities in epithelial cells in tobacco and betel quid chewers\textsuperscript{57}. Restriction of caloric intake can inhibit the formation of several types of experimental neoplasms, including oral cancer, and may have a significant effect in reducing morbidity in man\textsuperscript{58}. Kritchevsky reported that this is due to the fact that energy restriction moderates oxidative damage to DNA, enhances DNA repair and reduces oncogenes expression\textsuperscript{59}.

3.3.1 MALNUTRITION AND ORAL CANCER

Apart from inducing general immunosuppression, malnutrition may also impair salivary gland function and thereby reduce oral mucosal immunity. It is also associated with a significant reduction in the number of helper CD4+ cells and depressed natural killer cell activity. This suggests a breakdown in the capacity of the malnourished to mount effective tumour surveillance \textsuperscript{34}.

Franceschi et al suggested that high intake of particular dietary staples may be an indication of poor diet in general and that inadequate nutrition
enhances cancer risk. They opined that this may be due to the fact that dietary deficiencies are linked to high consumption of certain foods (for example maize is low in riboflavin)\textsuperscript{18}. Martinez in Puerto-Rico found that patients with oral, pharyngeal and esophageal cancers ate less food than did control subjects, were more likely to eat only one meal a day and were more likely to eat irregularly\textsuperscript{18}.

One of the earliest suggestions that nutrition may play a role in the aetiology of oral cancer comes from studies in Sweden that found a link
between iron deficiency anaemia (Plummer-Vinson syndrome) and pharyngeal cancer in women\textsuperscript{18}. Wynder \textit{et al} found that women with upper alimentary tract cancers were more likely than control subjects to have symptoms of the syndrome such as dysphagia and anaemia. The authors suggested that Iron deficiency exacerbated by poor diet was responsible\textsuperscript{61}. Rogers \textit{et al}, examining calcium, selenium, iron and zinc concentrations in toenail clippings of subjects, (this reflects nutrient concentrations 12-18 months in the past) found that lower concentrations of selenium in toenails was associated with increased risk of oral cancer. They however, observed no trend between oral cancer and decreasing toenail concentrations of Iron, calcium and zinc\textsuperscript{62}.

Many studies have found that people whose diets were deficient in fruits and vegetables were more likely to develop oral cancer. Winn \textit{et al} in a study in North Carolina USA showed that people who consumed 0-1 servings of fruits per week were significantly more likely to have oral cancer than those who consumed 7 or more servings per week\textsuperscript{18}.

Franco \textit{et al} in a study in Brazil also reported that people who consumed less than 1 serving of fruits per week were twice more likely to develop oral cancer than those who consumed 4 or more servings of fruits per week\textsuperscript{63}. Other studies have consistently shown an inverse relationship between fruit consumption and the risk of developing oral cancer\textsuperscript{64, 65}. Findings on vegetable consumption and development of oral cancer have
however, being less consistent. Gridley *et al* in an analysis of black subjects in the US observed that men with high total vegetable intake experienced 70% of the risk of those with low intake but they found no association for black women. Notani in India showed that those who do not eat vegetables daily had twice the risk of oral cancer as those with daily consumption. Franceschi found only a moderate protective effect of high vegetable consumption and suggested that chance may be the actual explanation for the differences observed. Day *et al* compared whites and blacks with respect to the extent and magnitude of risk associated with various health behaviours linked with oral cancer. They found that blacks had higher consumption of some fruits and vegetables, but the risk reduction associated with these factors was less in blacks than in whites. They suggested that the difference in race benefits of fruits and vegetables may be related to the difference in types of fruits and vegetables consumed, the method and duration of cooking and differences in cofactors related to risk in black and white populations.
3.3.2 ANTIOXIDANTS AND ORAL CANCER

Free radicals are molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. The unpaired electron(s) gives considerable reactivity to the free radicals. In biologic systems, two major groups of free radicals are noted; reactive oxygen species (ROS) and the reactive nitrogen species (NS). ROS and RNS are products of normal cellular metabolism and they have both beneficial and deleterious effects in living organisms. The deleterious effects that cause potential biological damage are termed oxidative stress and nitrosative stress respectively and occur in biologic systems when there is an over production of ROS/ RNS on one side and a deficiency of enzymatic and non enzymatic antioxidant activity on the other.

3.3.2.1 REACTIVE OXYGEN SPECIES (ROS)

Radicals derived from oxygen are considered to be the most important class of radicals generated in living systems. They can be beneficial in low to moderate concentrations when they are involved in defence against
infectious agents and some cellular signalling systems. However, at high concentrations, they are mediators of damage to cell structures, nucleic acids and proteins\textsuperscript{71}.

Examples of ROS are: supoxide anion (\(O_2^{-}\)), hydroxyl radical (\(\cdot OH\)) and the peroxyl radicals (ROO\(^{\scriptscriptstyle\cdot}\)). Supoxide is produced mainly in the mitochondria of cells. During the process of energy transduction, some electrons leak to oxygen prematurely, thereby, forming the supoxide free radical\textsuperscript{70}.

\[
O_2 + \text{NAD(P)H OXIDASES} \rightarrow O_2^{-}
\]
•OH + OH

9. Reactive Nitrogen Species (RNS)

3.3.2.2  REACTIVE NITROGEN SPECIES (RNS)

\[ \text{HOO}^+ \]
Free radicals are produced during normal oxidative cellular processes in the body. The production of free radicals is encouraged by ingestion of certain drugs, exposure to certain environmental factors such as smog, radiation, pesticides, herbicides, and habits such cigarette smoking.

Free radicals are highly unstable and if unchecked by antioxidants, are capable of damaging cell constituents, including DNA, as well as other opportune targets, particularly those containing polyunsaturated fatty acids. The increased incidence of cancer with advancing age may be partly due to the increasing level of free radical reactions with age coupled with the diminishing ability of immune system to eliminate altered cells.

Antioxidants are a wide variety of substances that play a vital role in mopping up free radicals and thus reducing or eliminating their potential for toxic cellular injuries. Examples of free radicals scavengers include superoxide dismutase, catalase, glutathione peroxidase, vitamins C and E, carotenoids, alpha-lipoic acid and bioflavinoids.

In a study of 69 children with various types of cancer, using children of same socio-economic status as control, Hal Nayel et al found no significant differences in serum levels of α-tocopherol and albumin in cancer patients and controls. They however, found serum retinol to be significantly lower in
patients with solid tumours than in patients with lymphoreticular neoplasm and primary brain tumours.\textsuperscript{74}

In a geographic region extending from Easter Iran to Southern Russia and parts of China, oropharyngeal cancers were found in people who do not appear to smoke and drink.\textsuperscript{75} There appears to be a dietary basis, mainly a rather poor nutritional intake of fruits and vegetables, which are major dietary sources of antioxidant nutrients.\textsuperscript{75} Beta-carotene was found to significantly inhibit experimental 7,12-dimethylbenz (a) anthracene induced squamous cell carcinoma of hamster buccal pouch.\textsuperscript{76} Most epidemiological studies on the relationship between oral cancers and vitamin C have demonstrated significant protective effect of increased vitamin C intake or fruit intake.\textsuperscript{17}

\textbf{3.4 PROTECTIVE ROLES OF ANTIOXIDANT VITAMINS IN CARCINOGENESIS}

Various studies have examined the roles of the antioxidant vitamins in the biology of cancer and some of the proposed mechanisms by which antioxidant vitamins may protect against cancer include:

\textbf{3.4.1 Vitamin A}

Vitamin A inhibits keratinization and terminal differentiation of epidermal cells. It also enhances cellular immunity by promoting an increase in number
of T-helper cells and NK cells. Furthermore, it helps in the arrest and reversal of leukoplakia progression. Vitamin A also induces cytotoxic and cytostatic effects on cancer cells and promotes apoptosis as well as interfering with cancer DNA and RNA gene expression. It is an anti-oxidant on singlet oxygen and stimulates tumour necrotic factor (TNF) and epidermal growth factor (EGF) activities.

3.4.2 Vitamin C

Vitamin C acts as an anti-oxidant, it also reduces vitamin E degradation and enhances chemo-taxis, phagocytosis and collagen synthesis. It inhibits nitrosamine formation and reduces oncogenes expression and circulating steroid levels.

3.4.3 Vitamin E

Vitamin E is a free radical scavenger, maintains membrane integrity, inhibits cancer cell growth and differentiation, mutagenicity and nitrosamine formation. Synergism between Vitamin E, selenium and ascorbate inhibits DNA and RNA protein synthesis in cancer cells.

3.4.4 Albumin

Albumin acts as an extracellular antioxidant and unlike antioxidant vitamins that scavenge reactive oxygen radicals; albumin scavenges mainly carbon-centered free radicals (C-radicals). These radicals can be involved in tissue
damage under hypoxic/anoxic conditions as well as in ischemia/reperfusion injury.

The various lines of evidence supporting a role of antioxidant vitamins in oral cancer prevention as suggested by Harinder includes epidemiological evidence, laboratory studies, effect on intermediate biomarkers, reversal of oral leukoplakia, and pharmacological evidence.

### 3.4.1 EPIDEMIOLOGICAL EVIDENCE

Epidemiological studies have consistently linked low intake of fruits and vegetables, which contain high vitamins A, C, and E levels, with increased risk of cancer, including head and neck cancers. The near unanimity of these studies constitutes one of the strongest lines of evidence of an etiologic linkage between diets and risk of cancer in the head and neck region. The nature of the evidence, which was derived from dietary estimates from structured questionnaires, however, does not allow definitive attribution of the benefit to a specific ingredient of fruit and vegetables.

There are diverse sources of antioxidants in the diet and these can be divided into non-nutrient antioxidants and the nutrient antioxidant. Flavonoids are the most important non-nutrient antioxidants and are mainly found in tea (black and green tea), red wine, onions and apples. The nutrient antioxidants are vitamin A, vitamin C and vitamin E. vitamin A is composed
of more than 600 carotenoids of which the β-carotene is the most important. Vitamin C is commonly found in citrus fruits (such as oranges, pineapple, and mango), peppers and potatoes. Good sources of β-carotene that are also readily available in the Nigerian community are fruits (such as mangoes, oranges), carrots, sweet potatoes and dark green leafy vegetables. Many fruits and vegetables that are readily available in Nigeria contain these nutrient antioxidants. Chima and Igyor in an analysis of nutrients in locally available vegetables in south-eastern part of Nigeria found that many of these vegetables had considerable amounts of Iron and vitamin C. They showed that six commonly consumed vegetables;
Because of the difficulty in measuring the amount of vitamin E in the diet, studies with this antioxidant are fewer in number but suggest protection. Epidemiologic studies suggest a significant lowering of oral cavity cancer
incidence, by as much as 50%, with the use of supplemental vitamins. These studies are important because they constitute some of the earliest epidemiologic evidence showing the need for supplemental Vitamin E for protective benefit \(^8^4\).

### 3.4.2 LABORATORY EVIDENCE

In laboratory studies, the carotenoids have been shown to have antimutagenic activity in bacterial systems. Similarly, in many cell culture systems, carotenoids prevent transformation induced by chemicals and radiation \(^8^5\). Of direct relevance to oral carcinogenesis are observations on the capacity of these compounds to block genotoxic damage in Chinese hamster ovary cells caused by oral carcinogens such as extracts of areca nut \(^8^6\).

An animal model of particular relevance to head and neck cancer is the hamster cheek pouch model in which precancerous and cancerous lesions are produced after application of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). This was first developed in 1954 by Salley and colleagues \(^8^7\). This tumour model is said to offer a more reliable gross and microscopic presentation of tumour retardation and prevention than systems based on transplanted tumors. The malignancies that develop are consistently carcinomas rather than sarcomas and there are associated
precancerous lesions. Other advantages of the hamster pouch model include; no spontaneously arising tumors that may complicate interpretation of effect of chemical carcinogen, untreated animals do not develop tumors and lesions so formed in hamster oral systems can be counted and measured, thereby, giving an overall approximation of tumor mass in various experimental groups of animals. Suda et al showed that retinoids (13- cis-retinoic acid and retinyl acetate) and β-carotene are active in inhibiting the formation of cancerous lesions in this system. Shklar et al and Weeraprdist et al showed that systemically administered vitamin E significantly inhibited the development of oral carcinogenesis in hamster pouch. Odukoya et al also showed that topically administered vitamin E produced similar results with systemically administered vitamin E in inhibiting oral carcinogenesis in the hamster pouch model. Using 48 female golden hamsters divided into 12 animals each, they found on gross examination, that animals whose buccal pouches were painted with DMBA but who also had topical vitamin E painted, demonstrated a significant delay in tumor formation in comparison with those who had only DMBA painted. They also found fewer and smaller tumors in the vitamin E treated animals and microscopic examination revealed smaller tumors with better cellular differentiation and less invasion.
They suggested that the ability of vitamin E to delay chemical carcinogenesis may be due in part to its antioxidant action which prevents formation of proximal carcinogens, thought to be diol epoxides. They also suggested another possible mechanism of action of vitamin E to be its ability to locally enhance immune response. Odukoya et al and Schwartz et al had in other studies showed that topical vitamin E stimulates the langerhans cells of buccal mucosa, resulting in greater number and altered morphology of dendritic processes.

3.4.3 INTERMEDIATE BIOMARKERS AND MICRONUCLEATED CELL FREQUENCY

Because of the difficulty of conducting prevention trials with cancer as the endpoint, there is considerable interest in developing intermediate endpoints that can function as markers for cancer risk. These usually are measurable histological, biochemical, genetic, or other markers that occur on the way to cancer development. When displayed, they place an individual at higher risk. Their modulation would be a way to assess the chemo preventive activity of
a test substance \textsuperscript{78, 92}. Several putative markers have been proposed; however, none has been proven to be fully validated \textsuperscript{92}. In vitro and animal model systems, β-carotene and vitamin E have been shown to modify many biochemical and genetic changes in the direction of inhibiting carcinogenesis, for example, increasing expression of the p53 suppressor gene \textsuperscript{93}. Increased frequency of micro nucleated cells may reflect genotoxic damage produced by carcinogens. Stich \textit{et al} reported that β-carotene, alone or in combination with vitamin A, can decrease the incidence of micro nucleated cells in exfoliated oral mucosal cells from populations considered to be at high risk for oral cancer \textsuperscript{94, 95, 96}.

### 3.4.4 REVERSAL OF ORAL LEUKOPLAKIA

The reversal or suppression of premalignant lesions is an important strategy against carcinogenesis for the prevention of cancer \textsuperscript{78}. The basis for this approach is that premalignant lesions are usually the first clinically identifiable clues that allow recognition of a mucosa that may be affected by carcinogenesis.

Table 1\textsuperscript{78} shows studies from different authors on the reversal of oral leukoplakia by oral administration of antioxidant vitamins.
In a study in India, Stitch \textit{et al} used a treatment protocol consisting of β-carotene (180 mg/wk, group 1), β-carotene plus vitamin A (100 000 IU/wk, group 2), or placebo (group 3) given twice weekly for 6 months. At 6 months, 15% of patients in group I, 27.5% in group 2, and 3% in group 3 had complete remissions of their lesions. Furthermore, the appearance of new lesions was strongly inhibited in the treatment groups\textsuperscript{96}.

In another study, using high dose vitamin A (200,000 IU) alone per week for 6 months, Stich \textit{et al}, reported a 57% complete response rate with complete suppression of new lesions after 6 months \textsuperscript{97}.

Benner \textit{et al} in a multicenter study reported a response rate of 46% in 43 subjects treated with 400 IU vitamin E twice daily for 6 months \textsuperscript{98}. Kaugars \textit{et al} in another study using a combination of antioxidant agents including β-carotene, α-tocopherol and vitamin C got a 56% response rate after 6 months\textsuperscript{99}.
<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>AGENT</th>
<th>COMPLETE RESPONSE</th>
<th>PARTIAL RESPONSE</th>
<th>OVERALL RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stitch (India)</td>
<td>β-carotene</td>
<td>15</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stitch (India)</td>
<td>β-carotene</td>
<td>27</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>+Vitamin A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garewell (USA)</td>
<td>β-carotene</td>
<td>8</td>
<td>63</td>
<td>71</td>
</tr>
<tr>
<td>Toma (Italy)</td>
<td>β-carotene</td>
<td>33</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td>Malaker (Canada)</td>
<td>β-carotene</td>
<td>28</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>_</td>
<td>_</td>
<td>56</td>
</tr>
<tr>
<td>Kuagers (USA)</td>
<td>+Vitamin C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+Vitamin E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benner (USA)</td>
<td>Vitamin E</td>
<td>23</td>
<td>23</td>
<td>46</td>
</tr>
</tbody>
</table>

NS- not specified
3.4.5 PHARMACOLOGICAL EVIDENCE

Many studies have shown that cigarette smokers have lower plasma concentrations of carotenoids than do nonsmokers\textsuperscript{100}. Stich \textit{et al} and Peng \textit{et al} found out that oral mucosal cells concentrations of β-carotene are lower in smokers than nonsmokers\textsuperscript{101, 102}. Peng \textit{et al} showed that this difference exists despite similar dietary intakes and that the magnitude of the difference is likely to be too large for smokers to achieve nonsmoker concentrations simply by diet modification\textsuperscript{102}. Kaugars \textit{et al} in cohort study, found lower dietary intake and lower plasma concentrations of carotenoids in those who subsequently developed premalignant lesions than those who did not, the observed difference was not statistically significant\textsuperscript{103, 104}.

In a case-control study conducted in Melbourne, Australia, Kune \textit{et al} using forty-one men with histologically confirmed oral or pharyngeal squamous cell carcinoma and 398 male community controls, found that the mean serum levels of β-carotene and vitamin A were statistically significantly lower in the cases than in controls\textsuperscript{19}.

In a study in Nagoya Japan, Nagao \textit{et al} using 48 oral leukoplakia (38males to 10 females) patients over the age of 40 years, found statistically significant lower serum levels of various antioxidants including β-carotene and vitamin E in males but no such
significant difference in the serum levels was observed for females \textsuperscript{22}. 
CHAPTER FOUR

JUSTIFICATION FOR STUDY

Oral cancer accounts for about 2% to 4% of all malignant tumours in most regions of the world. It still remains a very significant disorder both for the afflicted person and for the health care professionals. The trend for survival of patients with oral cancer is still rather disappointing over the past several decades. The overall 5-years survival rate of patients with oral cancer is put at about 50%.

Most authors accept tobacco and alcohol as the most important aetiological factors in the development of oral cancer. However, some authors have reported oral cancer in people who do not appear to use tobacco or consume any appreciable amounts of alcohol. This supposes that there is possibility of other important factors in the aetiology of oral cancer. This project is considered relevant due to the following reasons:

1. The need for more research on the aetiology of oral cancer
2. Paucity of published work on the relationship between oral cancer and nutrition especially antioxidant vitamins.
3. The need to update the general knowledge about oral cancer.
CHAPTER FIVE

METHODOLOGY

5.1 Design

This study was a case control study of serum albumin and antioxidant vitamins in oral epithelial cancer patients seen at Adeoyo state hospitals and UCH Ibadan.

5.2 Sample size

The sample size was calculated using the formula:

\[ n = \frac{Z^2 pq}{d^2} \]

Where \( n \) = the desired sample size

\( Z \) = the standard normal value set at 1.96

\( P \) = the proportion of target population estimated to have the condition. This was put at 2.0%, which is the estimated prevalence of oral cancer in Nigeria.\(^{23}\)

\( Q = 1.0 - p \)

\( D = \) the degree of accuracy desired which will be set at 0.05

Sample size \((n) = 1.96^2 \times 0.02 \times (1 - 0.02) / 0.05^2\)

\[ = 3.8416 \times 0.02 \times 0.98 / 0.0025 \]

\[ = 0.07529536 / 0.0025 \]

\[ = 30.118 \]
The sample was made up of 32 clinically diagnosed oral epithelial carcinoma patients and 30 normal patients (patients with no oral cancer, benign lesions and no known debilitating disease) serving as control. The oral epithelial cancer patients were seen in the dental clinic of the university College Hospital Ibadan over an 18 month period.

5.3 Ethical Clearance

Local ethical clearance was obtained from the ethical committee of University College Hospital, (UI/UCH –IRC). All patients were duly informed of procedure and informed consent form was signed by all patients who agreed to participate.

5.4 Selection Criteria

Inclusion Criteria

1. Subjects with clinically diagnosed oral epithelial cancer

2. Subjects free from both oral cancer and benign oral lesions were used as controls.

3. All subjects who consented to participate in the study (after signing consent form)
Exclusion Criteria

1. Clinically normal patients under age 40 years.

2. Patients who declined to participate in the study after due information on the content and procedure of study.

5.5 Administration of Questionnaire

A 33-item self administered questionnaire containing sections on bio-data, knowledge of oral cancer including nutritional and social habits was administered to 62 patients who consented to participate in the study (Appendix 2). The participants were divided into the following two groups:

Group 1 (n=32)……….. Oral epithelial Cancer patients

Group 2 (n=30)……….. Control patients

5.6 Clinical Examination

Oral examination was carried out on all patients. The examination was done under good illumination such as dental chair light using sterilized mirror and probes and wooden spatula. Adequate infection control procedures was ensured using gloves and facemasks

5.7 Histological Examination

Biopsy was performed in the Oral Surgery clinic of UCH by senior resident doctors under supervision of consultant surgeons, with standard surgical
techniques to include a 1cm margin of apparently normal mucosa in 32 clinically diagnosed oral cancer patients.

Biopsy specimens were fixed in 10% formalin and processed routinely in the Pathology laboratory. The paraffin blocks were sectioned at 3 microns thickness and stained with haematoxylin and eosin (H&E). All the H&E slides were examined by the researcher under supervision by a consultant in the department of Oral Pathology, UCH, to determine histological diagnosis.

5.8 Measurement techniques

Fasting blood samples of all participating patients were taken. The blood was centrifuged at 3,000 rpm for 5 minutes and separated serum was aspirated into tubes and analyzed for vitamins A, C and E and albumin with a DM520 spectrophotometer (Beckman USA).

5.8.1 Serum Vitamin A Measurement

This study measured serum Vitamin A using the spectrophotometric method described by Neeld and Pearson. This method uses trifluoroacetic acid to react with the conjugated double bonds of Vitamin A to form a faint blue compound. The colour change was assayed with a spectrophotometer.

5.8.2 Serum Vitamin C Measurement

In this study, Serum ascorbic acid was measured using the spectrophotometric method described by Roe and Kuether (1943) and
modified by Roe 1961. Ascorbic acid is converted to dehydroascorbic acid by shaking with Norit and this is then coupled with 2, 4-dinitrophenylhydrazine in the presence of fluorourea as a reducing agent. The dinitrophenylhydrazine thus formed is converted by sulphuric acid into a red compound, which can be assayed by spectrophotometer.

5.8.3 Serum Vitamin E measurement

Vitamin E activity is shown by 4 naturally occurring tocopherols α, β, δ and γ of which α-tocopherol is the most potent. Serum Vitamin E was measured by Emmerie-Engel reaction based on method described by Baker and Frank 1968. This method is based on the reduction by tocopherols of ferric acid to form ferrous ions which then forms a complex with α,α-dipyridyl, which was then assayed by spectrophotometry.

5.8.4 Serum Albumin Measurement

This was also assayed using the spectrophotometric method. Bromocresol Green (BCG) was reacted with the albumin in patients’ serum and the resultant green solution was assayed with spectrophotometer.

5.9 Data analysis

The data obtained was analyzed using SPSS Statistical Package (SPSS, Version 11.0). Difference between the two groups was analyzed for
statistical significance using the student t-test. Statistical significance was determined at P < 0.05.
CHAPTER SIX

RESULTS

From a total of 99 patients who fulfilled the inclusion criteria, 65 consented to participate while 34 patients did not consent to participate in this study, giving a response rate of 65.7%. Thirty (88.2%) out of the non-consenting patients were normal patients and 4(11.8%) had clinically diagnosed oral epithelial cancers. However, only samples from 62 respondents were analysed as the remaining three samples were lysed before getting to the laboratory.

From the 32 group 1 cases, 22(68.8%) were histologically diagnosed squamous cell carcinoma, 6(18.8%) adenocystic carcinoma, 3(9.3%) mucoepidermoid carcinoma and 1(3.1%) polymorphous low-grade adenocarcinoma.

6.2 Socio-demographic Profile of Respondents

The male to female ratio was 1:1.2. The overall mean age was 54.3 years (SD±14.5) while the age range was 18 to 83 years. The age range for males was 18 to 83 years while the mean age for males was 54.5 years (SD±15.7). The age range for females was 23-79 years and the mean age was 54.03 years (SD±13.6).
6.3 Age and Gender Distribution

The male to female ratio in the oral epithelial cancer group was 1:1.7. The age range was 18 to 83 years while the mean age was 53.7 years (SD±17.3). The peak age incidence for patients with oral cancer was the fifth decade accounting for 31.3% of total number of oral cancer seen (Figure 1a and 1b). Majority (81.3%) of the oral cancer cases occurred above 40 years of age.

The male: female ratio in the normal group was 1.3:1 and the mean age was 54.8 years (SD±10.9) while the age range was 40-78 years with 60% of them being above age of fifty.

Student t test showed there was no statistically significant difference between the mean ages of patients with oral cancer and the control group. (p = 0.788, t = 0.270, C.I. = - 6.337 to +8.308).
Figure 1a- Age group distribution of patients (%)
Fig 1b- AGE GROUP DISTRIBUTION (FREQUENCY)
6.4 Educational Qualification

In the oral epithelial cancer group, 23.3% had no formal education while 80% of all patients in the oral epithelial cancer group had below university education. Oral epithelial cancer group had the highest percentage (23.3%) without any formal education. The sharpest drop in percentage of patients with increasing level of education was seen in oral epithelial cancer group as compared with the normal group (Fig 2). However, student t test showed no statistically significant difference in the level of education between the two groups (p = 0.304).
Figure 2- EDUCATIONAL QUALIFICATION OF PATIENTS
6.5 Occupation

In this study, oral epithelial cancer occurred most frequently in traders (34.4%) followed by farmers, civil servants and artisans with each group accounting for (12.5%) of cases seen (Table 2). Other occupations such as students, engineers, housewives, retired and unemployed occurred occasionally altogether accounting for (12.4%) of cases. Trading (26%) and artisans (26.7%) were the most frequent occupation in the control patients. Student t test shows that there was a statistically significant difference in the occupational distribution of patients (p=. 00).
Table 2: Occupation of patients

<table>
<thead>
<tr>
<th>OCCUPATION</th>
<th>NORMAL</th>
<th>MALIGNANT</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Civil servant</td>
<td>3 (10.0%)</td>
<td>4 (12.5%)</td>
<td>7 (16.0%)</td>
</tr>
<tr>
<td>Student</td>
<td>1 (3.3%)</td>
<td>1 (3.1%)</td>
<td>2 (5.3%)</td>
</tr>
<tr>
<td>Artisan</td>
<td>8 (26.7%)</td>
<td>4 (12.5%)</td>
<td>12 (20.2%)</td>
</tr>
<tr>
<td>Trading</td>
<td>8 (26.7%)</td>
<td>11 (34.4%)</td>
<td>19 (30.9%)</td>
</tr>
<tr>
<td>Retired</td>
<td>7 (23.3%)</td>
<td>1 (3.1%)</td>
<td>8 (8.5%)</td>
</tr>
<tr>
<td>Housewife</td>
<td>3 (10.0%)</td>
<td>3 (9.4%)</td>
<td>6 (6.4%)</td>
</tr>
<tr>
<td>Clergy</td>
<td>0 (0.0%)</td>
<td>2 (6.3%)</td>
<td>2 (2.1%)</td>
</tr>
<tr>
<td>Engineering</td>
<td>0 (0.0%)</td>
<td>1 (3.1%)</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>Farming</td>
<td>0 (0.0%)</td>
<td>4 (12.5%)</td>
<td>4 (7.4%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>0 (0.0%)</td>
<td>1 (3.1%)</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>32 (100.0%)</td>
<td>62</td>
</tr>
</tbody>
</table>

(100.0%) (100.0%)
6.6 Income per Annum

Oral epithelial cancer occurred more commonly in patients in the lower income groups, with a total of 79.3% of oral epithelial cancer patients earning 50,000 Naira or less per annum. Only 13.7% earn above 50,000 Naira per annum. In the high-income group of 500,000 Naira per annum and above, only one patient (3.4%) was seen.

40% of the control patients earn 50,000 Naira or less, while up to 60% earn 50,000 Naira and above. However none of the patients in the control group earn up to 500,000 Naira.

Figure 3 shows a bi-modal peak income for oral epithelial cancer patients in the income groups of 0-10,000 Naira and 20,000-50,000 Naira while the control patients featured a peak income of 50,000 to 200,000 Naira. There was also a sharp drop in the proportion of oral epithelial cancer cases as income increased to 50,000 Naira level. There was however a delay in the drop until income level was above 200,000 Naira among control group.

Student t test shows a statistically significant difference in the incomes of the oral cancer respondents and the control group of respondents (p=0.018).
Figure 3- Income per annum of patients
6.7 Aetiological Factors

6.7.1 Tobacco

Only 23.1% of the oral epithelial cancer patients in this study use tobacco, however, this was much higher than the 6.9% who use tobacco in the control group (Table 3). There was, however no statistically significant difference in tobacco use between the oral cancer group and the control (p= 0.106).

6.7.2 Alcohol

Most people in this study do not take alcohol and only 25.8% of oral epithelial cancer cases reported that they take alcohol (Table 3) and 24.1% of the normal group take alcohol. There was no statistically significant difference in alcohol use between the oral cancer patients and the control (p=0.884).

6.7.3 Diet

All the respondents (100%) in the oral cancer group reported that they take vegetables as part of their diet; only one (3.4%) of the control group does not take vegetables (Table 3). However, 32.3% of oral cancer, and 3.6% of the control do not take fruit as part of their diet. There was no statistically significant difference in vegetable consumption between the oral cancer patients and control patients (p=0.305). However, there was a statistically
significant difference in fruit consumption between the oral cancer patients and the control patients (p=0.004)
### Table 3: Aetiological factors

<table>
<thead>
<tr>
<th>Aetiological factors</th>
<th>Normal No (%)</th>
<th>Oral cancer (%)</th>
<th>Total No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Tobacco use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (6.9)</td>
<td>6 (23.1)</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>No</td>
<td>27 (93.1)</td>
<td>20 (76.9)</td>
<td>47 (88.1)</td>
</tr>
<tr>
<td>Total</td>
<td>29 (100)</td>
<td>26 (100)</td>
<td>55 (100)</td>
</tr>
<tr>
<td><strong>2. Alcohol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (24.1)</td>
<td>8 (25.8)</td>
<td>15 (21.1)</td>
</tr>
<tr>
<td>No</td>
<td>22 (75.9)</td>
<td>23 (74.2)</td>
<td>45 (79.9)</td>
</tr>
<tr>
<td>Total</td>
<td>29 (100)</td>
<td>31 (100)</td>
<td>60 (100)</td>
</tr>
<tr>
<td><strong>3. Vegetable</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (6.9)</td>
<td>6 (23.1)</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>No</td>
<td>27 (93.1)</td>
<td>20 (76.9)</td>
<td>47 (88.1)</td>
</tr>
<tr>
<td>Total</td>
<td>29 (100)</td>
<td>26 (100)</td>
<td>55 (100)</td>
</tr>
</tbody>
</table>

xxxiv
### Consumption

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28(96.6)</td>
<td>1(3.4)</td>
<td>29(100)</td>
</tr>
<tr>
<td></td>
<td>31(100)</td>
<td>0(0)</td>
<td>31(100)</td>
</tr>
<tr>
<td></td>
<td>59(96.6)</td>
<td>1(3.4)</td>
<td>60(100)</td>
</tr>
</tbody>
</table>

### Fruit consumption

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27(96.4)</td>
<td>1(3.6)</td>
<td>28(100)</td>
</tr>
<tr>
<td></td>
<td>21(67.7)</td>
<td>10(33.3)</td>
<td>31(100)</td>
</tr>
<tr>
<td></td>
<td>48(84.3)</td>
<td>1(15.7)</td>
<td>49(100)</td>
</tr>
</tbody>
</table>
6.8 Serum Antioxidants

6.8.1 Serum Albumin

The mean serum albumin level was lower in the oral epithelial cancer patients with a value of 3.5g/dl (SD±0.7) while the control group had a higher mean serum albumin level of 4.5g/dl (SD ± 0.49).

Student t test showed a statistically significant difference in the mean serum levels between the oral epithelial cancer group and control patients (p=.000, f=1.13) (C.I 0.19-0.76). Figure 4 shows 83.3% of normal patients had serum albumin levels within normal (3.5-5.0g/dL) while only 45.2% of the oral epithelial cancer patients were within the normal range. More oral epithelial cancer cases (51.6%) had below the lower limit of normal serum levels of albumin (<3.5g/dL) compared with only 3.3% of control patients who had below the lower limit of normal.
Fig 4: Serum Albumin of Patients
6.8.2 Serum Vitamin A

The mean serum level of Vitamin A was lower in the oral epithelial cancer group 0.53mcg/L (SD±0.26) compared with 0.81mcg/L (SD±0.28) in control patients. There was a statistically significant difference in the mean serum levels of vitamin A between the control and oral cancer groups (p=.022, f=1.88) (C.I. -0.27-0.25). Oral epithelial cancer had less percentage of respondents (58.1%) with serum levels within the normal range for serum vitamin A (normal range 0.5-2.0mcg/L) compared to the control group that had 91.3% within normal values of vitamin A (Figure 5). Only 6.7% of the control group had below the lower limit of normal serum vitamin A (<0.5mcg/L) compared with 41.9% of oral cancer respondents having below the lower limit of normal serum vitamin A.
Fig 5: Serum vitamin A of Patients
6.8.3 Serum Ascorbic Acid

The mean serum level of ascorbic acid in the oral epithelial cancer group was 0.63mg/dl (SD±0.28) and the control group had a mean serum level of 0.91mg/dl (SD±0.25). Student t test showed statistically significant difference in mean serum ascorbic acid levels between the oral cancer and the control group of respondents (p=.000,f=0.46) (C.I = 0.31-0.30). Figure 6 shows only 3.3% of control patients had serum levels below the lower limit of normal (<0.5mg/dL) while 25.8% of oral cancer group had serum levels less than 0.5mg/dL.
**Fig 6: Serum vitamin C of Patients**
6.8.4 Serum Vitamin E

The mean serum level of Vitamin E in the oral epithelial cancer patients was 6.68mg/L (SD±3.03) which was less than the 8.80mg/L (SD±3.48) in the control patients. Student t test showed a significant difference in mean serum level of vitamin E between the control and oral cancer groups of respondents (p=.013, t=3.57) (C.I = -0.39-3.57). Figure 7 shows most patients in the two groups had serum levels of Vitamin E less than the lower limit of normal (<10mcg/L). However while a total of 36.7% of the normal patients had normal or elevated levels of serum vitamin E, only 9.7% of oral cancer group had normal or elevated levels of serum Vitamin E.
Fig 7: Serum vitamin E of Patients
Test of significance within the different age groups shows that there was a statistically significant difference in mean serum albumin levels only in the 40-49 years age group (p=0.003). There was a statistically significant difference in serum Vitamin A and Vitamin E only in the 40-49 years age group (p=0.006) and (p=0.013) respectively. However, there was a statistically significant difference in the mean serum Vitamin C levels in age groups 50-59, 60-69 and above 70 years with (p= 0.048), (p=0.024) and (p=0.025) respectively (Table 4).
Table 4: Test of significance of serum indices within Age groups

<table>
<thead>
<tr>
<th>AGE GROUP</th>
<th>ALBUMIN</th>
<th>VIT A</th>
<th>VIT C</th>
<th>VIT E</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>0.596</td>
<td>0.47</td>
<td>0.127</td>
<td>0.732</td>
</tr>
<tr>
<td>30-39</td>
<td>0.514</td>
<td>0.61</td>
<td>0.498</td>
<td>0.619</td>
</tr>
<tr>
<td>40-49</td>
<td>7.534</td>
<td>0.003</td>
<td>6.166</td>
<td>0.006</td>
</tr>
<tr>
<td>50-59</td>
<td>4.229</td>
<td>0.056</td>
<td>2.370</td>
<td>0.155</td>
</tr>
<tr>
<td>60-69</td>
<td>2.647</td>
<td>0.104</td>
<td>0.215</td>
<td>0.809</td>
</tr>
<tr>
<td>70+</td>
<td>2.253</td>
<td>0.172</td>
<td>1.402</td>
<td>0.270</td>
</tr>
</tbody>
</table>
6.9 Socio-demographic Factors and Serum Indices

6.9.1 Gender

Table 5 shows consistently higher mean serum levels of albumin, vitamin A, C and E in males compared with females. Independent samples t test however shows no statistically significant difference in mean serum indices between males and females.
Table 5: COMPARISON OF SERUM ALBUMIN AND ANTIOXIDANT VITAMINS ACCORDING TO GENDER.

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Mean</th>
<th>P-Value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>4.15 ± 0.69</td>
<td>0.19</td>
<td>Not significant</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>3.89 ± 0.82</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>0.78 ± 0.28</td>
<td>0.88</td>
<td>Not significant</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>0.76 ± 0.31</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>7.48 ± 3.70</td>
<td>0.49</td>
<td>Not significant</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>7.93 ± 3.15</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>0.67 ± 0.31</td>
<td>0.11</td>
<td>Not significant</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>0.67 ± 0.30</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>
6.9.2 Income

Table 6 shows no statistically significant difference in serum indices with respect to income of control and oral cancer groups.
Table 6: COMPARISON OF SERUM ALBUMIN AND ANTIOXIDANT VITAMINS ACCORDING TO INCOME

<table>
<thead>
<tr>
<th></th>
<th>MEAN</th>
<th>F-VALUE</th>
<th>P-VALUE</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.381</td>
<td>0.738</td>
<td>0.600</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.144</td>
<td>1.141</td>
<td>0.168</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.110</td>
<td>1.413</td>
<td>0.237</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>7.53</td>
<td>0.580</td>
<td>0.715</td>
<td>Not significant</td>
</tr>
</tbody>
</table>
6.9.3 Educational Qualification

Table 7 shows no statistically significant difference in serum albumin and antioxidant vitamins with respect to educational qualification of oral cancer and control patients.
Table 7: COMPARISON OF SERUM ALBUMIN AND ANTIOXIDANT VITAMINS ACCORDING TO EDUCATIONAL STATUS

<table>
<thead>
<tr>
<th></th>
<th>Mean square</th>
<th>f-value</th>
<th>p-value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.391</td>
<td>0.652</td>
<td>0.652</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.095</td>
<td>1.039</td>
<td>0.252</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.137</td>
<td>1.620</td>
<td>0.183</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>15.433</td>
<td>1.347</td>
<td>0.265</td>
<td>Not significant</td>
</tr>
</tbody>
</table>
6.9.4 Occupation

Table 8 shows no statistically significant difference in serum albumin and antioxidant vitamins with respect to occupation of oral cancer and control patients.
Table 8: COMPARISON OF SERUM ALBUMIN AND ANTIOXIDANT VITAMINS ACCORDING TO OCCUPATION

<table>
<thead>
<tr>
<th></th>
<th>Mean square</th>
<th>f-value</th>
<th>p-value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.680</td>
<td>1.171</td>
<td>0.334</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.083</td>
<td>0.900</td>
<td>0.532</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.115</td>
<td>1.389</td>
<td>0.218</td>
<td>Not significant</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>15.179</td>
<td>1.408</td>
<td>0.209</td>
<td>Not significant</td>
</tr>
</tbody>
</table>
CHAPTER SEVEN

DISCUSSION

Although oral cancer accounts for about 2% to 4% of all malignant tumours in most regions of the world, it still remains a very significant disorder both for the afflicted person and for the health care professionals. The trend for survival of patients with oral cancer is still rather disappointing over the past several decades. The overall 5 years survival rates of patients with oral cancer are put at about 50%.

Most authors accept tobacco and alcohol as the most important aetiological factors in the development of oral cancer. However, some authors have reported oral cancer in people who do not appear to use tobacco or consume any appreciable amounts of alcohol.

Suda et al showed that topically applied beta-carotene inhibited experimental oral carcinogenesis in hamster pouch. Other studies have shown that diets high in fruits and vegetables, vitamin A and vitamin C have protective effect against oral cancer. However some other studies claimed no benefit from antioxidant vitamins supplements in the occurrence of oral cancer.

The age range for oral carcinoma patients in this study was 18 to 83 years with a mean age of 53.7 years. This compares favourably with studies by
Arotiba et al in Ibadan, Ajayi et al in Lagos and Otoh et al in Maiduguri that reported a mean ages of 53.7 years, 51 years and 56.5 years respectively. This was however higher than the mean age of 48 years reported by Daramola et al and Lawoyin et al both in Ibadan and 49 years by Odukoya et al in Lagos. Pinhort in Denmark and Krutchkoff in Connecticut United States, reported mean ages of 69 years and 62.4 years respectively.

The peak age incidence of oral carcinoma patients in this study was in the fifth decade (40 to 49 years), which was lower than some studies that reported peak incidence in the seventh decade (60 to 69 years). 81.3% of oral carcinoma patients in this study were above 40 years of age while only 18.7% were below 40 years of age. This is similar to many African studies such as that of Chidzonga in Zimbabwe and Ajayi in Lagos who reported 70.8% and 75% respectively for of oral carcinomas occurring above the age of 40 years. However, Lawoyin et al reported that 92.3% of oral cancer in Ibadan occurred above age 40 years. Sugerman found 95% of oral cancer cases in Australia to be above 45 years of age. Many other Caucasian studies also reported occurrence of higher percentages of oral carcinoma patients above the age of 40. The lower percentage of oral cancer occurring above the age of 40 years in this study and other
African studies compared to Caucasian studies may be due to the lower life expectancy and or early exposure to risk factors\textsuperscript{112}. The percentage of people under 40 years is much higher and life expectancy (47 years) is much lower in the Nigerian population than in the US\textsuperscript{39}. There was higher preponderance of oral cancer among female patients in this study with a male to female ratio of 1 to 1.7. This is at variance with many previous studies that reported a higher male preponderance with authors reporting male to female ratios of 1.7 to 1, 1.5 to 1 and 1.5 to 1 in Ibadan and 1.5 to 1 in Lagos\textsuperscript{30, 39}. Studies in England, United States and Denmark also reported higher male preponderance\textsuperscript{109, 113, 114}. However, Van Wyk et al, found a higher female preponderance in South African Indians with male to female ratio of 1 to 1.6. They attributed this to the fact that areca nut chewing was more common in South African Indian women than men\textsuperscript{115}. Many authors have reported a declining trend in the male to female ratio of oral cancer and have explained this by the fact that oral cancer incidence in women is increasing due to increased tobacco use by women\textsuperscript{6, 109, 114}. The higher female preponderance in this study may be due to the small sample size and the small percentage of respondents who use tobacco. Studies on the association of socio-economic status and oral cancer have been somewhat conflicting\textsuperscript{116}. Some studies reported no association
between oral cancer and education and occupation, while others showed a decreased risk of oral cancer with higher socio economic status based on occupation and higher levels of education.¹¹⁷,¹¹⁸,¹¹⁹

In this study there was an inverse relationship between the occurrence of oral cancer and education with 80% of oral cancer cases having less than a university education. This is similar to a study by Williams who showed college education had an inverse relationship with lip and tongue cancer.¹¹⁹

Hashibe in a study in India observed that a higher percentage of patients presenting with oral leukoplakia were in the lower education group and majority of female cases were without any formal Education.¹²⁰

There was an inverse relationship between oral cancer and income in this study, with most patients (79.3%) earning 50,000 Naira or less per annum. This finding is similar to that of Hashibe in India, which showed that oral premalignant lesions were commoner in people with low household income, as those earning less than 1,500 rupees had the highest incidence of oral premalignant lesions, while those who earn more than 3,000 rupees had the lowest incidence of the lesion.¹¹⁶

However, Hodell et al, in a review of oral cancer data from 172 countries showed that income and other socio economic factors do not appear to be a strong factor in the worldwide incidence of oral cancer.¹²¹,¹²²
Various studies have reported varying relationships between occupational exposures and the risk of oral cancer. Some authors reported high risk of oral cancer amongst plumbers, welders, metal workers, painters and electrical workers and attributed this to exposure to high level of metal dust and solvents.\textsuperscript{123, 124, 125, 126} In this study, trading was the most predominant occupation of oral cancer respondents, accounting for 34.4\% of oral cancer cases. This was followed by artisans and civil servants. The high percentage of traders presenting with oral cancer in this study may be due to the fact that many of them are petty traders who may be more in the low socio-economic class, which are prone to nutritional deficiencies.

Hashibe \textit{et al} in a study in India showed that people in the lower socio-economic groups were more likely to use tobacco and drink alcohol.\textsuperscript{111} Other authors also reported an inverse relationship between tobacco use, alcohol drinking and socio-economic status.\textsuperscript{117, 118, 122} Kerr \textit{et al}, in a United States study, reported that in addition to higher prevalence of alcohol and tobacco use, people of lower socio economic groups were more likely to consume less fruits and vegetables, a finding that was similar to that of Hashibe in India.\textsuperscript{116, 121}
Only 26.1% of oral cancer cases in this study use tobacco in any form while 25.8% use alcohol and 12.5% reported they use both tobacco and alcohol. Arotiba et al reported that 49% of patients presenting with oral squamous cell carcinoma in Ibadan had positive history of predisposing factors such as tobacco and alcohol use. Most other studies have shown higher percentage of tobacco use. Blot et al, in a study in the United States, found that 75% of oral cancer cases were associated with tobacco smoking and heavy alcohol use. Lissowaka et al, reported that 82% of oral cancer cases in Poland reported that they use tobacco compared to 65% in the control group. Sugerman reported that 75% of oral cancer cases in Australia were associated with smoking and alcohol. Other studies in Denmark and Brazil reported that 86% and 63.9% respectively of oral cancer cases in their studies use alcohol and tobacco. The low percentage of oral carcinoma patients presenting with alcohol and tobacco use may be due in part to the reluctance of some of the patients volunteering history of alcohol and tobacco.

In this study, 100% of the oral cancer group claim to take vegetables as part of their diet while 96.6% of the normal patients take vegetables as part of their diet. However, 20% of oral cancer patients take vegetable with every meal, compared with 25% of control group that take vegetables with every meal. Only 67.7% of oral cancer patients take fruits as part of their diet.
compared with 96.4% of control patients that take fruits as part of their diet. Furthermore, 9.5% of oral cancer patients take fruits after every meal while 18.5% of the control group that take fruits after every meal.

McLaughlin et al, found that those in the lower quartile of fruit intake had a risk ratio of 1.7 for men and 2.0 for women, while Winn et al found that those who consumed fruit once per week or less had 1.7 times the risk of those who consume fruit seven times a week or more\textsuperscript{18,130}. Winn reported that seven studies found no association between oral cancer and vegetable intake\textsuperscript{18}. Cindley et al, observed that men with high vegetable intake experience 70% of the risk of those with low intake but no association was found in women\textsuperscript{130}. Francescin et al, in a study in Italy observed that only selected vegetables such as carrots, fresh tomatoes and green peppers had protective effect for oral cancer\textsuperscript{65}. In a study in India, Notani et al showed that those who did not eat vegetables had twice the risk of oral cancer as those with daily consumption\textsuperscript{131}.

The relatively high percentage of oral carcinoma patients that consume vegetables in this study may be explained by the fact that though many people take vegetables, they may not get maximum benefit from the vegetables due to the common habit of overcooking vegetables, which may denature the vitamins.
The mean serum level of albumin for oral epithelial cancer in this study was statistically significantly lower than for controls. There is a paucity of studies on the relationship between serum albumin and oral cancer. Knekt et al, in a study in Finland observed an elevated risk of cancer of the distal colon at higher concentration of Serum Albumin. They however, found no significant association between serum albumin and cancer of proximal colon and rectum\textsuperscript{132}. Ko et al, however, reported a 60\% reduction in colon cancer risk in the highest serum albumin quartile in comparison to lowest quartile\textsuperscript{133}. However, Glattre et al, found no significant reduction in thyroid cancer risk with increasing serum level of albumin and suggested increased risk of follicular type of carcinoma of thyroid with increasing serum albumin levels\textsuperscript{134}. The low levels of serum albumin in oral carcinoma patients in this study may be due to the presence of inflammatory mediators IL-6 and TNF produced by tumour and host cells in malignancies. Inflammatory mediators increase the transcapillary escape rate of albumin such that albumin is lost to the tissue. Inflammatory mediators also decrease the production rate of albumin\textsuperscript{135}.

In this study, mean serum levels of vitamin A, C and E were significantly lower in oral carcinoma cases compared to the control. Most studies are in agreement with this finding. Abiaka et al, found $\alpha$-tocopherol concentration
to be significantly lower in stomach, colon, rectal and breast cancer cases compared to controls\textsuperscript{136}. Choi \textit{et al}, found that the serum level of ascorbic acid in gastric carcinoma patients in Seoul was less than one-fifth of their control. They also found that serum levels of beta-Carotene and alphatocopherol of gastric cancer patients were significantly decreased compared to their control group\textsuperscript{137}. Other studies have consistently showed significantly lower serum levels of antioxidant vitamins in lung, bladder, breast and prostate cancer patients \textsuperscript{138,139,140,141}

Zheng \textit{et al}, in Maryland United States, observed that serum level of beta-carotene were lower in subjects that subsequently developed oral and pharyngeal cancer\textsuperscript{21}. Ramaswamy \textit{et al}, found that serum levels of vitamin A and C were significantly higher in oral leukoplakia cases compared to controls. However, no significant difference was observed in the serum levels of vitamin E in oral leukoplakia cases compared to controls\textsuperscript{20}. This was also corroborated by Kune \textit{et al} who observed in a study in Australia that serum vitamin A was significantly lower in oral and pharyngeal cancer cases compared to controls\textsuperscript{18}. However, Nagao \textit{et al}, in a study in Japan found serum $\beta$-carotene to be significantly lower in oral leukoplakia than controls in males, but in females, no significant difference was noted in the
serum α-tocopherol and β-carotene levels in leukoplakia patients compared to controls\textsuperscript{22}.

It may be argued that the low serum levels of vitamin A, C and E in patients with oral epithelial cancers may be due to low or improper consumption of vitamin containing foods thereby reducing the protective effects of antioxidants against cancer\textsuperscript{39}. The low serum levels may also be due to loss of appetite that may be caused by Tumour necrosis Factor (TNF) and IL-6 produced in cancer patients\textsuperscript{135}. Consequently, there is general malnutrition, including reduced intake of vitamins.

This study was not able to categorically determine whether the lower serum levels of antioxidants in oral cancer patients relative to controls was due to malnutrition resulting from oral cancer or that the low serum levels predisposed the patients to oral cancer.
CHAPTER EIGHT

CONCLUSION

It is concluded from this study that:

1. Oral cancer occurred more commonly in individuals who are 40 years and above in this series.

2. Tobacco and alcohol do not appear to be strong factors in the aetiology of oral cancer in this study.

3. The mean serum levels of albumin, vitamin A, vitamin C and vitamin E were lower in oral epithelial cancer patients than in control group.

4. There was an inverse relationship between education and oral cancer occurrence, also between income and occurrence of oral cancer.
CHAPTER NINE

LIMITATIONS OF STUDY

The following are considered the limitations of this study:

1. This study was not able to categorically conclude whether the low levels of serum albumin and antioxidant vitamins predisposed to oral cancer, or poor nutrition secondary to oral cancer lead to the low serum values.

2. The relatively small sample size may limit interpretation of results from this study.

3. Dearth of published works on this subject, especially in this part of the world made it quite difficult to have an extensive comparison of the findings in this study with other studies.
CHAPTER TEN

RECOMMENDATIONS

It is recommended that:

1. More studies examining the relationship between nutrition and oral cancer need to be carried out especially in West African sub-region where there is a dearth of literature on this aspect of oral cancer research.

2. Similar case control studies using larger sample size are recommended.

3. Nutritional and dietary counselling regarding the importance of fruits and vegetables need to be re-emphasised in the community oral health education programmes.
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APPENDIX 1- PRO-FORMA

SERUM ALBUMIN AND ANTIOXIDANTS IN ORAL CANCERS

Patient’s Name: 
Hospital No.: 
Address: 
Marital Status: 
Age: 
Sex: 
Occupation: 
Examination: 
IOE: 
Location: 
Size: 
Differential Diagnosis: 

Serum Albumin 
Serum vitamin A 
Serum vitamin C 
Serum vitamin E
APPENDIX 2-

QUESTIONNAIRE

I will be grateful if you careful fill this questionnaire, as it will greatly help us in fulfilling the purpose of this study. Your answers shall be treated with the strictest confidentiality.

Please do answer the questions sincerely and appropriately.

Thank you for your co-operation.

SOCIO-DEMOGRAPHIC DATA

1. Age:

2. Sex: Male ☐ Female ☐

   b. Married ☐ d. Widowed ☐

4. Religion a. Christianity ☐ c. Traditional ☐
   b. Islam ☐ d. Others Specify________

5. Tribe a. Yoruba ☐ c. Ibo ☐
   b. Hausa ☐ d. Others specify__________

6. Educational Qualification
   a. None ☐ b. Secondary School ☐
   b. Primary School ☐ d. Graduate ☐ Post Graduate ☐

7. Occupation a. Civil Servant ➤ Student ☐
c. Artisan ☐ d. Others specify____________

8. Income per annum
   a. - 0 -10,000 ☐ b. - 20,000-50,000 ☐
   c. - 10,000 – 20,000 ☐ d. - 50,000 – 200,000 ☐
   e. - 200,000 – 500,000 ☐ f. - Above 500,000 ☐

KNOWLEDGE ABOUT ORAL CANCER

9. Have you ever heard about oral cancer?
   Yes ☐ No ☐

10. If yes, how did you hear about it?
    1. From Family members / friends ☐
    2. From my family physician ☐
    3. From a dentist ☐
    4. Workshop / Seminar ☐
    5. Media – Radio, TV, Newspaper ☐
    6. Others (Specify)……………………

11. Which of the following in your opinion can cause oral cancer?

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Strong Agree</th>
<th>Agree</th>
<th>Disagree</th>
<th>Strongly Disagree</th>
<th>I don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Kola nut</th>
<th>Sunlight Radiation</th>
<th>Smoking / Tobacco</th>
<th>Malnutrition</th>
<th>Bad Oral hygiene</th>
<th>Worms</th>
<th>Hole in your tooth</th>
<th>Influence of Evil</th>
<th>Spirits</th>
<th>Inheritance form</th>
<th>Parents</th>
</tr>
</thead>
</table>

12. Do you eat green leafy vegetables as part of your diet at all?
   - Yes □
   - No □

13. If yes, how do you eat it?
   a. Raw □
   b. Cooked □

14. If cooked, for how long
   a. 2min □
   b. 4mins □

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15. If yes, how many times.
   a. At least once a day
   b. At least 2 times a week
   c. At least 3 times a week
   d. At least 5 times a week

16. Do you take fruits as part of your diet at all?
   Yes ☐ No ☐

17. If yes, how many times?
   a. After every meal
   b. At least five times a week
   c. At least thrice a week
   d. At least twice a week

SOCIAL HABIT ASSESSMENT

18. Do you take tobacco in any form? Yes ☐ No ☐

19. If yes, what form.
   a. cigarette Smoking  ☐   b. Pipe smoking  ☐
   c. Snuff  ☐   d. Tobacco Chewing  ☐

20. How often do you use tobacco?

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21. For how long have you been using tobacco?
   a. About 6 months ago  
   b. More than 1 year ago  
   c. More than 3 years  
   d. More than 5 years  
   e. More than 10 years  

22. Do you take alcohol in any form?  
   Yes  
   No  

23. If yes, please state in what form
   a. Beer  
   b. Alcoholic Spirits  
   c. Alcoholic wine  
   d. Local Brews  

24. How many bottles do you take in a day?
   a. More than 3 Bottle per day  
   At least one bottle per day  
   c. 3 bottles per week  
   d. At least one bottle per week  
   e. Occasionally  

25. For how long have you been taking alcohol?
   a. For about 6 months  
   b. More than one year  
   c. More than 5 years  
   d. More than 10 years  

26. Do you eat Kola nut?  
   Yes  
   No  

27. If yes, how many nuts do you take?
a. More that 3 nuts per day   □. At least one nut per day

c.         nut in per week   □   d. one nut per week

 e. □casionally   □

28. For how long have you been taking kola nut?

 a. for about 6 months   □   b. for more one year

□ for about 5 years   □   d. For about 10 years   □

29 Who referred you here for treatment?

 a. Medical Doctor   □   b. Dentist   □   c. Nurse   □

 d. Health Officer   □   e. Others (please state)_____

30. What treatment were you given before referral?

A No treatment   □   b. Oral Drugs   □

c. I.V. Drugs   □   e. Others (Please state)_____

31. For how long were you treated before referral?

 a. Immediately   □   b. After 3 days   □   c. After 1 Week   □

d. After 2 weeks   □    e. After 1 month   □

32. How long did you wait before presenting

 a. Immediately   □   b. After 3 day   □   c. After 1 week   □

d. After 2 weeks   □   e. After 1 Month   e. After 3 months

33. Has any member of your family had cancer before?

Yes   □    No   □
34. If yes, which member of your family?
   a. Mother  
   b. father  
   c. brother  
   d. Sister  
   e. uncle/ aunts  
   f. cousin  

35. What type of cancer did the above have?
   a. Oral cancer  
   b. breast cancer  
   c. prostate cancer  
   d. Stomach cancer  
   e. skin cancer  
   f. eye cancer  

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APPENDIX 3

INFORMED CONSENT FORM

My name is Lawal Ahmed Oluwatoyin. I am a Resident Doctor of the Department of Oral Pathology (College of Medicine, Faculty of Dentistry), University College Hospital, Ibadan.

I am carrying out a study on The Relationship of Malnutrition and Oral Cancer in Patients attending Dental Centre, U.C.H. In the study, blood samples will be taken from a vein and biopsy will be done for histological confirmation.

You are free to take part in this study. You have the right to withdraw at any given time if you choose to.

Consent: Now that the study has been well explained to me and I fully understand the consent of the study process, I will be willing to take part in the study.

........................................... ...........................................
Signature of Participant &Date Signature of Investigator & Date
UI/UCH INSTITUTIONAL REVIEW COMMITTEE

CERTIFICATION LETTER

Principal Investigator: Dr. Lawal Ahmed Oluwatoyin

IRC Protocol No: UI/IRC/06/0010

Protocol Title: CORRELATION OF SERUM ALBUMIN AND ANTIOXIDANT VITAMINS IN ORAL CANCER PATIENTS IN IBADAN.

STATUS: APPROVED

The UI/UCH Institutional Review Committee has reviewed your protocol titled: “Correlation of Serum Albumin and Antioxidant Vitamins in Oral Cancer Patients in Ibadan.”

The proposal is set out to establish the relationship between serum albumin and antioxidant vitamin levels and oral cancers among dental outpatients of the University College Hospital and Adeoyo State Hospital, Ibadan.

THE RESEARCH PROTOCOL DESCRIBED ABOVE HAS BEEN REVIEWED BY THE UI/UCH IRC WITH THE RESULTS AS INDICATED.

F. A. A. Adeniyi
Professor/Chair, UI/UCH IRC
E-mail: uiuchirc@yahoo.com

International Regulations require that any severe drug reactions and unexpected adverse occurrence to subjects during the conduct of this research be reported to the UI/UCH IRC Secretariat promptly. Any changes to this protocol must be submitted for review to the UI/UCH IRC.
APPENDIX 5

SPECTROPHOTOMETER
APPENDIX 6

SQUAMOUS CELL CARCINOMA OF THE LOWER LIP
APPENDIX 7

ADENOCYSTIC CARCINOMA OF THE PALATE