A study investigating the etiological association between oral cancer and use of chewstick in the Sudan

By
Dr. Ali Mohamed Idries

A thesis submitted for the degree of Doctor of Philosophy (Ph.D).

Department of Pathology, Faculty of Medicine,
University of Khartoum
1982
Dedication

To the soul of my late brother Sherif a victim of this deadly disease.
Acknowledgement

I would like to express my thanks to my Internal Supervisor Professor Mohamed Osman Abdel Malik, Vice Chancellor of Ain Shams University and Chairman of Radio Medical Research Council for his valuable assistance and encouragement. My thanks and appreciation also go to my external supervisor Dr. Peter Morgan of the Department of Oral Pathology and Medicine, Guy's Hospital, London for guidance and support.

I also wish to acknowledge that a part of this thesis was completed during my sabbatical leave to University of Washington, Seattle, USA. I wish to acknowledge, the help of Professor M. Weiss, Head Department of Epidemiology, and Associate Professor Elaine Faustman, Department of Environmental Health, School of Public Health and community Medicine, University of Washington, and Professor David Thomas Head of Unit of Epidemiology, at the Fred Hutchinson Cancer Research Center, at Seattle, State of Washington, USA.

My thanks are also to Dr. Jack Estevé Chief Unit of Biostatistics and information, Dr. H. Barbier Chief Unit of Environmental Carcinogenesis and Host Factors and their colleagues, at the International Agency for Research on Cancer (IARC), Lyon, France for their help in the chemical analysis of tobacco.
Abstract

In the first part of this study (toombak) was defined, history of its introduction to the Sudan was studied, its agricultural aspects, fermentation, processing and curing were studied. The aims behind study of historic background of toombak, were to define and draw attention to the use of toombak in Sudan. Literature on history and use of various forms of smokeless tobacco was reviewed. Information on the prevalence of toombak usage among Sudanese people was provided. The species of toombak plant was identified for the first time. Definition and properties of tobacco as major additive were studied. Its chemistry, trace elements composition, radioactivity were investigated. Methods of processing of toombak, current practices of the habit of toombak, production use of toombak.

The second part of the study was based on descriptive epidemiology of patients with oral cancer. Data from 1916 cases of oral neoplasms occurring over a 15-year period, from January 1970 to December 1985, were retrieved and analyzed. The study revealed that the frequency of oral neoplasms is relatively high when compared with neighboring countries and accounts for about 7.6% of all neoplasms of the pathological sites studied. However, this figure may in fact be an underestimate of the real frequency for the various reasons noted. Squamous cell carcinoma was the component of the oral malignancies (66.6%) followed by salivary gland neoplasms (14.6%) odontogenic neoplasms (8.6%) and neoplasms of other histological types (10.3). Males have a higher frequency
Tobacco taken in the form of snuff is believed to play a major role in the etiology of oral squamous cell carcinoma in the Sudan.

The third part was based on analytic epidemiology of patients with oral squamous cell carcinomas. The material was obtained from hospital records. The possible association of squamous cell carcinomas of the lip, buccal cavity, and floor of mouth and the use of toombak was investigated retrospectively. History of use of toombak in 350 patients (first group of cases) with squamous cell carcinomas of these sites, 271 patients (second group of cases) with squamous carcinomas of the tongue, palate and masticatory muscles, were compared with 204 patients (first group of controls) with non-squamous oral and non-oral neoplasms and 2820 individuals (second group of controls) selected from the general population. Using stepwise logistic regression analysis, controlling simultaneously for age, sex, and tribe, strong association was found between squamous cell carcinomas of the lip, buccal cavity, and floor of the mouth. Among long duration users the relative risk reached 7-fold. The relative risk reached 27-fold among non-smokers who used toombak for more than 10 years. The elevated risk found when using cancers of introral sites with little or no direct contact with toombak as controls confirms the hypotheses that direct contact with tissues in
An important mechanism in tobacco carcinogenesis is the mouth.

The fourth part was based on another analytical epidemiology. The study was undertaken to examine the risk associated with use of tobbak using data obtained by direct and more detailed interviews of cases and controls.

A case control study involving 114 cases with oral squamous cell carcinoma and 93 hospital based controls was carried out. The study investigated the risk related to usage of tobbak, smoking of cigarettes, and consumption of alcohol. After controlling for age, sex, residence, smoking of cigarettes, and alcohol consumption, a stepwise logistic regression yielded a relative risk associated with tobbak usage of 5.9 (95 per cent confidence limits 2.3-15.3).

Among chronic tobbak users the relative risk was 10.7 (95 per cent confidence limits 6.4-114.7). The relative risk increased 27-fold (95 per cent confidence limits 6.4-114.7) among users who retain the tobbak suits for longer durations in the mouth. A relative risk of 4.9 (95 per cent confidence limits 1.0-22.9) was found among alcohol users only. Smoking did not increase the relative risk among tobbak users. Increased relative risk associated with cigarette smoking among non-tobbak users was observed.

The risk attributable to use of tobbak was 60.2 per cent. Thus, about two third of the cases of oral cancer in the Sudan were associated with tobbak use. It is expected that elimination of the habit of tobbak use would reduce oral cancer by two third in the Sudan.
Since previous and above epidemiological studies suggested an association between tobacco use and subsequent oral cancer development, it was important to characterize analytically the nitrosamine content of this type of snuff. Therefore, the fifth part of this study was analysis of toombak and saliva of toombak users for tobacco specific nitrosamines (TSNA). TSNA levels in 20 samples of Sudanese toombak, of different quality, collected from five different vendors, and 12 samples of saliva obtained from toombak users were analyzed. Using gas chromatography coupled with thermal energy analysis, four TSNA were quantified in snuff extracts: N-nitrosocornamoline (NNN), N'-nitrosonornabasine (NAB) and 4-(methyl nitrosamine)-1-(3-pyridyl)-1-butanone (NNK). Unusually high levels of these TSNA (mean; range (mg/g snuff, dry wt)) were detected: NNN (1.13; 0.50-3.00); NAB (0.08;0.02-0.23); NNK (0.22;0.02 2.37); and NMR(2.31; 0.62-7.87). Previously, the highest levels of NNN and NNK reported in any snuff were 0.134 mg/g; dry wt and 0.014 mg/g dry wt, respectively. In comparison, the levels in Sudanese toombak were up 20 and 500 times higher, respectively.

Levels of N-nitrosocornamoline (NNN), N'-nitrosornabasine (NAB), N'-nitrosonornabasine (NAB) and 4-methyl nitrosamine-1-(3-pyridyl)-1-butanone (NNK) were also measured in saliva of toombak users before, during and after toombak taking. In addition, two TCNA, 4-(methyl nitrosamine)-1-(3-pyridyl)-1-butanone (NNAL), were detected for the first time and were confirmed by gas chromatography.
Nine out of 10 subjects had detectable saliva levels of total TSNA before chewing (0.01-1.0), and immediately following chewing (0.1-2.0) μg/ml. During dipping TSNA concentrations reached μg/ml levels: (range, μg/ml: number of subjects positive) NNI (0.0-3.1; 12/12), NAT (0.0-0.9; 2/12), NAB (0.5-1.8; 12/12), RKN (0.0-0.6; 11/12) NRI (0.0-3.3; 11/12) and imo-NRNI (0.0-0.4; 8/12). These saliva TSNA levels, which are 10-100 times the levels previously reported, are consistent with above observations of unusually high TSNA in Sudanese khatab.

The evidence accumulating from above epidemiologic and laboratory studies is strong that khatab can cause oral cancer in humans. The association between oral cancer and use of khatab are strongest for cancers of the lip, buccal and floor of mouth. The risk is several times greater among chronic khatab users. The potential carcinogen identified in khatab suggest that khatab may cause cancers in other organs, however, evidence for cancers outside the mouth are little.

It is expected that these findings would draw attention to khatab use as a significant public health hazard, and stimulate efforts to reduce exposure to TSNA in khatab users in the Sudan.
لا يمكنني قراءة النص العربي من الصورة المقدمة. إذا كنت تحتاج إلى مساعدة في شيء آخر، فأبلغني بذلك.
المقدمة
لا ينبغي النظر في أمور الإجهاض كعنصر مهم في المشاكل الصحية والاجتماعية، بل يجب النظر إليها كأحدkpعوامل المرتبطة بالعنصر الاجتماعي والاقتصادي. وهو ما أدى إلى زيادة نسب الإجهاض، مما يشكل صعوبة كبيرة في مواجهة هذه المشاكل. خصوصًا في مجتمعات متخلفة، حيث يتم فرض القوانين الدينية والتشريعية على المرأة والطفل، مما يمنعهم من التفاعل الاجتماعي والاقتصادي.

من ناحية أخرى، فإن الإجهاض يعتبر من أكثر القضايا الملموسة في العالم، حيث تتأثر العديد من البلدان بقضايا الإجهاض، مما يسفر عن تأثيرات سلبية على الأسرة ومجتمع الإنسان بشكل عام.

لا يمكننا الإجابة على الأسئلة الملموسة بشأن كيف يمكننا القضاء على هذه المشاكل، ولكن من المهم أن نلاحظ أن الحلول تشمل مجموعة واسعة من الاستراتيجيات، بما في ذلك التعليم، الصحة، والتنمية الاجتماعية.

التعليم
التعليم هو أداة قوية لحل هذه المشاكل، حيث يمكننا استخدام التعليم كى نشجع الناس على الإجراءات الملحقة، وتوفر لهم المعلومات التي ستساعدهم على أخذ قرارات مستنيرة. هذا يشمل تعزيز المعرفة حول الرضاعة الطبيعية، وعدم الإجهاض، والرعاية الصحية العامة.

الصحة
الصحة هي مادة أساسية للحياة، حيث يتم التركيز على الرعاية الصحية في جميع المستويات، من خلال توفير الرعاية الصحية العامة والчастية، والتعليم الصحي، والدعم الاجتماعي. هذا يساعد على تقليل نسبة المعدومين، وتعزيز نجاح الأسرة، وتعزيز الصحة العامة.

التنمية الاجتماعية
التنمية الاجتماعية هي أيضًا جزءًا مهمًا من الحلول، حيث يتم التركيز على تحسين الظروف الاجتماعية والاقتصادية، والمساهمة في تحسين الظروف الحياتية للعديد من الأشخاص. هذا يشمل توفير الفرصة للتعليم، فرص العمل، والدعم الاجتماعي، وتحسين الرعاية الصحية العامة.

للمزيد من المعلومات حول هذه المقالة، يرجى زيارة الموقع الرسمي للمؤسسة المختلطة للتعليم والصحة.
عدد من هذه المواد في لعبة اللعب الذي يستعملونه من 10 إلى 100 مرة.
الانعكاسات العقلية والاعتبارات الأنسانية لديهم دور في تأكيد أن التنبؤ بسبب
السرطان الفم ولكن العلاقة البعيدة بين السمنة وسرطان الفم والشغف وثبات الفسيم
الدوري برهانًا مباشراً بعجز الفم الأخرى وزيادة الفرق في احتفال الأصدقاء كلما
تبادلت مجموعة استخدام المواد وتجرد الإشارة إلى أن المواد السرطانية التي يحتوي عليها
الشام تسبب السرطان في أنسجة وأجزاء أخرى من الجسم ولكل من
الأيام في هذا المجال قليلة. يتوقع أن يكون الفحص الذي تجدها في هذا
البحث دوماً في النص العلمي بأن استخدام المواد ذو أثر ضعيف بحجم
المجتمع لأن تؤثر هذه النتائج إلى تحويل جرعة المسبب بالصحة للつつيف من تكرار
الإنسان لتحسينات السمان السرطانية.
Contents

Title .................................................................................. 2
Dedication ........................................................................... 11
Acknowledgement.............................................................. 13
Abstract (English) ............................................................... vi
Abstract (Arabic) ............................................................... vii
Table of contents .............................................................. xii
List of tables ........................................................................ xiv
List of Photographs & Figures ............................................ xv
Glossary ............................................................................. xvi

1 Chapter One (General Introduction and Objectives) ...... 1
  1.1 General Introduction .................................................. 2
  1.2 Objectives .................................................................. 10

2 Chapter Two (Studies of Tuberculosis) ......................... 14
  2.1 Introduction ................................................................ 15
  2.2 Review of Literature .................................................. 19
  2.3 Material and Methods ............................................... 25
  2.4 Results ...................................................................... 33
  2.5 Discussion .................................................................. 55

3 Chapter Three (Descriptive Epidemiology) ................. 59
  3.1 Introduction ................................................................ 60
  3.2 Review of the Literature ............................................ 61
  3.3 Material and Methods ............................................... 83
  3.4 Results ...................................................................... 87
  3.5 Discussion .................................................................. 91

4 Chapter Four (Analytical Epidemiology) ...................... 99
  4.1 Introduction ................................................................ 101
  4.2 Review of the Literature ............................................ 102
  4.3 Material and Methods ............................................... 124
  4.4 Materials and Methods .............................................. 113
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chemistry of Atrow</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>Elemental Contents of atrow</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td>Analysis of atrow for trace elements</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>Cancer Registries in Africa</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>Age Standardized Cancer Incidence Rate: Africa</td>
<td>71</td>
</tr>
<tr>
<td>6</td>
<td>Age Standardized Cancer Rate: Asia</td>
<td>74</td>
</tr>
<tr>
<td>7</td>
<td>Histological Types of Oral Neoplasms</td>
<td>102</td>
</tr>
<tr>
<td>8</td>
<td>Case Control Characteristics</td>
<td>125</td>
</tr>
<tr>
<td>9</td>
<td>Relative Risk Estimates</td>
<td>128</td>
</tr>
<tr>
<td>10</td>
<td>Case Control Characteristics</td>
<td>130</td>
</tr>
<tr>
<td>11</td>
<td>Relative Risk Estimates</td>
<td>131</td>
</tr>
<tr>
<td>12</td>
<td>Adjusted Relative Risk Estimates</td>
<td>128</td>
</tr>
<tr>
<td>13</td>
<td>RR Adjusted For Interactions</td>
<td>135</td>
</tr>
<tr>
<td>14</td>
<td>Levels of TSMA in Tomboh</td>
<td>151</td>
</tr>
<tr>
<td>15</td>
<td>Levels of TSMA in saliva of Tomboh users</td>
<td>153</td>
</tr>
</tbody>
</table>
List of Photographs & Figures

Photo 1: Plantation of tobacco
Photo 2: Tobacco flowering
Photo 3: Tobacco field
Photo 4: Harvesting of tobacco (Culayab)
Photo 5: Autoradiograph Atron Tested for radioactivity
Photo 6: Fermentation of tobacco
Photo 7: Tobacco prepared in large bags for export
Photo 8: Tobacco processing
Photo 9: Tobacco ready for sale
Photo 10: Tobacco sellers
Photo 11: Tobacco quid "naffa"
Photo 12: Tobacco qid (quid) in lower lip
Photo 13: Lower lip lesion
Figure 1: Districts for tobacco cultivation
Figure 2: Age Standardized Cancer Rates: Africa
Figure 3: Distribution of age by types of oral tumours
Figure 4: Sex Distribution of Various Types
Figure 5: Distribution of various tumours by tribe
Figure 6: Distribution of various tumours by residence
Figure 7: Percentage of TSNA in Tobacco
Figure 8: A chromatogram for TSNA in Tobacco
Figure 9: A chromatogram for TSNA in saliva
Figure 10: Mass spectrum for Tobacco
Glossary

IARC : International Agency for Research On Cancer
ICD-3  : International Classification of Disease Eighth Revision
ICD-o  : International Classification of Diseases for Oncology
ASCAR : Age Standardized Cancer Rate
SICA : St. John and Impole Centre, Everton
SCR   : Sudan Cancer Registry
WHO  : World Health Organization
Chapter 1
General Introduction and Objectives
Cancer was generally thought to be a problem of the Western World since the reported incidence indicated that it was much rarer in most developing countries. Hence, although it has received the attention of health planners in most of the developed countries by contrast little attention was paid to it in developing countries. Current reports on the occurrence of cancer, however, suggest that it constitutes a worldwide problem. Although it has been estimated that over one half of the annual world total of 8.8 million new cases occur in developing countries (Parkin and Muir, 1984) there is increasing need for data on current trends in cancer incidence, morbidity, and mortality in the developing countries. The present scarcity of data on cancer incidence in the developing world (Waterhouse et al, 1976, 1982) is due to a number of factors, including lack of cancer services, lack of cancer registries, and the lack of facilitation for research (Parkin and Muir, 1984).

Evidence derived from epidemiological studies suggests that at least 50% of cancer cases are due to environmental rather than genetic factors (Higginton, 1979). The need for identification of such environmental factors has accentuated the need for the study of disease occurrence in different geographical locations, population groups, and time periods.

Support given by the World Health Organization (WHO) and other International Organizations to developing countries to establish their own cancer registries has resulted in useful
data on cancer occurrence. Recently, information on the occurrence of cancer in developing countries has been compiled in two comprehensive monographs: "Cancer Occurrence in Developing Countries" (Parkin and Harries 1984) and "Cancer Prevention in Developing Countries" (Khagali et al. 1986). Data provided in these publications indicate a much higher frequency of cancer in developing countries than was previously reported. More precise measures of this disease are needed to assess the extent of the cancer problem and to provide clues as to the etiology of the different cancers.

Cancer is currently the fourth most common health problem in the Sudan according to available mortality figures (Kass, 1990) ranking behind infectious diseases, cardiovascular disease, and road accidents. Although cancer is thus recognized as an important health problem, it has not yet received due attention from health workers and planners.

Available information on cancer from the Sudan consists of a few reports on the relative frequency of certain types of cancer. Dickey (1958) provided information on the relative frequency of epithelial malignant tumors in Sudanese. Then a number of reports on cancer followed in the 1960s. These reports are the following: Lynch et al. (1965) investigated Cancer in general in the Sudan. The most common tumors were skin (18.5%), the female genital organs (14.6%), the breast (11.2%), and the salivary glands were (6.1%). Pattern of surgical diseases in two areas of Sudan were described by (Nabi 1965); he noted the difference in malignant neoplasms in Juba in the South and Port Sudan in the east. He remarked on the high frequency of Burkitt's

It is recognized that the risk of cancer at specific anatomical sites exhibits remarkable geographic variation throughout the world. Greater risk differences may be identified if incidence rates are derived for subpopulations on the basis of ethnicity, occupation, socio-economic characteristics, and customs and habits. Cancer affecting individual organs or anatomical sites comprises a variety of histomorphological types and exhibits significant differences in epidemiology, aetiology, and behaviour.

Oral malignant tumours have no uniform nomenclature, (Fildes 1936). Since the initial numerical definition established by the International Classification of Disease (ICD) (World Health Organization 1977), the designated numbers for specific sites of the oral cavity have been a subject of continuous revision (editions: 7th, 1957; 8th, 1967 and 9th, 1977).

Although rare in most developed countries, oral cancer constitutes a major health problem in many developing countries (Parkin and Muir, 1984). In spite of this fact the incidence of cancer in Africa is low (Fildes, 1936). However, the highest rates for oral cancer were reported among the peoples of south and Southeast Asia (Bangladesh, Burma, Kampuchea, India, Malaysia, Nepal, Pakistan, Singapore, Sri Lanka, Thailand, and Viet Nam) and to thought
to be related to the habit of chewing tobacco containing products. (HRH, 1980). In some parts of India, it accounts for approximately 40 per cent of all cancers. (Piekarcz 1990).

Tobacco has been established as an important epidemiological factor for cancer affecting several body sites, including oral cancer (Parkin and Mar 1984). Clinical and epidemiological reports have consistently made reference to the relatively high frequency of oral cancer among smokers of tobacco or tobacco-containing products, and snuff users. Such data have been reviewed by several study groups (WHO 1984; International Agency for Research on Cancer; IARC 1985; The Surgeon General, USA 1986; WHO 1988). A number of case-control studies in the USA have shown that the risk is several times greater among snuff users than among nonusers (Moore et al 1987; Kubo et al 1993; Nylander et al 1987; Reznick et al 1985; Wewer et al 1982; Vincent and Marshott 1983; Williams and Horn 1981; Wits et al 1981). Other case-control studies on snuff use and oral cancer have been done in Sweden (Nylander et al 1987), the United Kingdom (Brown et al 1977; Paul Rico (Markides 1990).

Case-control studies have provided further evidence of increased risk of oral cancer among snuff users (Raklis and Herman 1980; Finn et al 1982; Smith et al 1970.). The IARC (1985) emphasized the evaluation of the clinical data from USA and western Europe by the following statement: "There is sufficient evidence that use of snuff of the type commonly used in America and Western Europe is carcinogenic in humans. There is limited evidence that chewing tobacco of the type commonly used in these areas is carcinogenic.
Epidemiological studies that did not distinguish between chewing tobacco and snuff provide sufficient evidence for carcinogenicity of oral use of smokeless tobacco products as reported in these studies. In aggregate, there is sufficient evidence that the use of smokeless tobacco of the above types is carcinogenic to humans. There is sufficient evidence that oral use of tobacco mixed with lime (khaini) is carcinogenic to humans. There is inadequate evidence that oral use of the other smokeless-tobacco preparations considered (naswar, nasuwar, mishri, gumkhala and shehnau) are carcinogenic to humans. There is inadequate evidence to evaluate the carcinogenicity of chewing tobacco, snuff or mass to experimental animals. The Surgeon General, (USA 1986) and (WHO 1988) evaluated data on snuff and chewing tobacco and reached similar conclusions.

The relative risk of oral cancer is increased among people with habits of chewing tobacco containing products in Asia. Case control studies from India have arrived at such conclusions, although a wide variation was found in the magnitude of the risk encoutered. However, the relative risk remained high among chewers of tobacco containing products (Per 1973; Ravhavi 1975; Anand 1975; Narang 1985; Sahu & Karkamkarathi 1985, 1987; Wahi 1975; Panneerselvam et al 1979; Mandal & Gangadh 1976; Jayanti et al 1977; Sahu et al 1976). Results of cohort studies were reported by Wahi (1980) and Mohle et al (1972). The consistent findings that oral cancer develops more frequently among users of tobacco products placed provides further evidence for a causal relationship between tobacco and oral cancer.
A positive-dose response relationship and duration of use between the tobacco quid habit and oral cancer was also found (Orr 1983; Hirosema 1982; Winn et al 1981, 1982; WHO 1984). IARC (1985) evaluated data from Asia and other parts of the world by the following statement. "There is sufficient evidence that the habit of chewing betel quid containing tobacco is carcinogenic to humans. There is inadequate evidence that the habit of chewing betel without tobacco is carcinogenic to humans. The working group also concluded that, while there is sufficient evidence that the combined habits of smoking tobacco and chewing betel quid without tobacco cause oral pharyngeal cancer, the evidence considered here does not allow an assessment of the possible contribution of betel quid without tobacco to this carcinogenic risk. There is limited evidence that areca nut with or without tobacco is carcinogenic to experimental animals. Data is inadequate to allow evaluation of the carcinogenicity of betel leaf or areca nut to experimental animals". The Surgeon General, USA (1986) and WHO (1988) arrived at similar conclusions.

The use of tobacco containing products in a form of snuff for chewing, has also been shown to increase the risk for cancer of other sites including the larynx, pharynx, esophagus, oropharynx, base of the tongue, and hypopharynx (Sastry 1985; Sinha 1985; Khanafer 1984; Wynder et al. 1961, 1977; Martinez 1988; Datta & Krishna-murthy 1987; Steven & Uragoda 1970). Additional sites include the pancreas, urinary tract, nasal cavity, and stomach.
The relationship between oral use of smokeless tobacco products and precancerous lesions, such as leukoplakia, lichen planus, and submucous fibrosis, is widely reported in the literature (Flindberg 1983; Gupta 1980; TARC 1985). Other adverse health effects relate to dental tissues such as gingival recession, soft tissue changes at the site of placement of the tobacco, degenerative changes of the salivary gland, cardiovascular and digestive effects, fetal toxicity, and addiction and dependence (TARC 1985; Surgeon General, USA 1988; WHO 1988).

Snuff used in Western Europe and North America has been shown to contain human carcinogens such as volatile aldehydes, polynuclear aromatic hydrocarbons (PAH), potassium 210 and volatile N-nitrosamines all of which have been found to be associated with increased risk for oral cancer (Hoffman & Adams 1981; Hoffman et al. 1988). The high amounts of Tobacco Specific N-nitrosamines (TSNA) found in smokeless tobacco (Hoffman and Hecht 1985) are considered to be among the likely causative agents for the cancer of upper alimentary tract, lung, urinary and bladder cancer in tobacco users (Bartlach & Mantzara 1964; Hoffman & Hecht 1985; Hecht & Hoffman 1984). Of the 24 N-nitroso compounds that have been identified in smokeless tobacco products, TSNA derived from nicotine alkaloids contribute 70-80% of the total amount (Tricker & Froehlichmann 1989). TSNAs consist of N-nitrosornicotine (NNN) and
of these, NBR and MHR, are strongly animal carcinogens even at low doses (Hoffman and Heckl 1982). The IARC evaluated data on carcinogenicity of NBR, MBR, and NAT. No case reports or epidemiological study of carcinogenicity of these chemical compounds in humans was available. They concluded that "there is sufficient evidence for carcinogenicity of NBR and MBR in experimental animals. The available data are inadequate to evaluate the carcinogenicity of NAT in experimental animals."

Williams (1988) was the first to report an elevated frequency of oral cancer in the Sudan. El-Hassan (1982) studied the relative frequency of various cancers from the biopsy reports from the National Health Laboratory and made observations that the relative frequency of oral cancer decreased as the number of biopsies increased from non-accessible sites. He concluded that the earlier reported high frequency of oral cancer in biopsied apicectomies was due to site accessibility. Friend et al. (1968) made similar remarks and stated that the high relative frequency of oral cancer was due to its site accessibility, ease of tooth extraction, and poor dental hygiene. They stated that oral surgery conditions were also common, thus 50% of all cases were in the Sudan. Other studies have also suggested an association between oral cancer and tobacco use. Haidar (1962), Houch et al. (1983), El-Moshty et al. (1991) reported the relationship of smokers and oral cancer in the Sudan. They found that 50 out of 62 patients with oral carcinoma were smokers. They developed a "coffee" mask and that often developed at the site where the mask was placed.
The primary purpose of this study is to provide information on the use and effects of various forms of nicotine in the Sudan. The specific aim is to provide biological evidence in support of the potential role of Sudanese tobacco in the causation of lung cancer in the Sudan. Criteria for such evidence for carcinogenicity will be derived from descriptive and controlled studies of the population of the Sudan, laboratory tests on the products used to estimate the levels of tobacco-specific nitrosamines (TSNA), and studies to estimate the levels of TSNA in the diets and urine of smokers.

1. Study of Tobacco

The study will provide information on the tobacco in the Sudan, including historical overviews, cultivation and farming practices, botanical characteristics, methods of drying, processing, production, and use. Information will also be provided concerning the physical and chemical characteristics, locations, production, and use of tobacco. Toxicity of tobacco and its components in the body will be investigated.

2. Descriptive Study

This study will provide information on the relative frequency of malignant neoplasms, particularly those of the oral cavity, in the Sudan. It will examine the geographical distribution of oral cancer within the Sudan and explore differences in the
pattern of cancer between that country and its neighbors using published data from existing tumor registries. Specifically, it will determine the relative frequency of oral cancer as a proportion of all malignancies, the relative frequencies of various oral malignancies, and examine the data for differences in age, sex, ethnic groups, and/or geographical distribution.

3. Case Control Study

The specific aims of the case control studies are:

1) To assess differences in toombak use among cases and controls;
2) To assess differences in smoking and/or alcohol use among cases and controls;
3) To assess case control differences regarding previously suggested risk factors for oral cancer, so that the independent effects on risk of toombak can be determined and any interactions identified;
4) To examine the following hypotheses:
   a) Use of large quantities and/or increased frequency of use of toombak are associated with increased risk of squamous cell carcinoma of the habitual and buccal mucosa.
   b) Smoking increases the risk of squamous cell carcinoma of the tongue, palate and maxillary sinus.
   c) Alcohol consumption increases the risk of oral cancer more than the additive risk of either that of toombak or alcohol alone.
   d) That the rate of oral cancer is correlated with the intensity and/or frequency of exposure to toombak, smoking, and alcohol.
That overall exposure to khanbak, cooking, and smoking was associated with elevated risk of oral cancer.

Studies on carcinogenicity

The oral use of beedleums tobacco, either in the form of snuff (as used in North America and Western Europe) or with lime in the form of a betel quid (as used in Asia) has been unequivocally associated with human cancer (IARC 1985). However, little has been published about beedleums tobacco as it is used in the Sudan and other African and Middle Eastern countries and how its use is associated with neoplastic disease. The main tobacco species are Nicotiana rustica and N. tabacum (locally called khanbak). The leaves are mixed with an aqueous solution of natrium hydrobromide-tartrate, an alternative base to slaked lime, and in other parts of the world by tobacco chewers until it becomes saturated and set. In the Sudan, the prevalent oral use of beedleums tobacco is in the form of khanbak prepared from raw dried leaves mixed with natrium (El-Heehir 1988).

The present study was undertaken to investigate the exposure to TMA in Khanbak in the Sudan and permit evaluation of the role of these nitrates in the incidence of oral cancer. Snuff and saliva samples were collected and analyzed for TMA and nitrites.

The toxicity of natrium was evaluated by analyzing its chemical and elemental composition. Levels present in humans were analyzed from saliva and urine samples of people.
As previously stated, the study will examine other factors which may increase or decrease the risk of oral cancer and confound associations of interest. In particular, cultural and socioeconomic factors such as tribal identity, occupation, and geographical area of residence will be addressed. Carcinogenicity studies of the Sudanese tobacco plant and potential toxicity of niatam will help to shed light if specific toxic agent(s) in is/are responsible for the malignancies commonly found in regular users of tobabak.
Chapter 2
Studies of Toumbak
Unprocessed tobacco, mixed with additives and flavoring agents, i.e. sniff and betel nut chewing, are given local names in different parts of the world. These products vary according to type of tobacco used and additives incorporated. The pattern associated with their use, and availability of products also vary among different nations (IARC 1985; The Surgeon General, USA 1980).

In Asia and the USA, tobacco is sometimes chewed by itself without any additives being used (IARC 1985, The Surgeon General, USA 1980). However, in some parts of the world tobacco may not be included in the chew or may form only a minor component. In parts of New Guinea, for example, arnica root is usually chewed directly, and lime is scraped on the oral mucosa, tobacco being rarely added (Hastie et al 1988). In Indonesia and Malaysia, lime and betel leaf are chewed, after which the betel and tobacco are masticated with tobacco (Hastie et al 1988).

The leaf of betel (Piper betle) is the primary leaf, which is most commonly of the Areaceae family. The leaf is dried in the sun for 1 or 2 days, and is then rolled up into a tube, or filled with tobacco or lime. In Java, the leaves are used as a garnish in leaf-wrapped meat dishes (Hastie et al 1988).
an advance plan to which Tomahawk was to be incorporated.

In addition, many used in the formation of Tomahawk

The main interest in Tomahawk was directed to
its possible causal relationship with cold cancer. A few
records from the Sudan, dealt with some aspect of possible

The Sudan, covering an area of one million square miles,
is the largest country in the African continent. The
geography and ethnography of the country was described by

It was estimated that the population of the Sudan in 1990
was 26,000,000 with a population growth rate of 3.0% per
year (Hassan 1988). 75% of the population were urban, 71.7
male, and 11.6 female. About one third of the population
lives in Khartoum, the capital city, and the central region
of the Sudan. 20% live in the southern region, 10% in the
northwest, and 20% in the southern region.

In the northwestern region of the Sudan, the
major urban centers are Khartoum, the capital. The
major river in this area is the Nile River. There are
many historical sites and monuments in the
region, including the Pyramids of Giza in Egypt,
which have been an important source of ancient
Indian civilization.

The history of the use of Tomahawk in the Sudan
region has been well documented.
The major undertaking of this report is to define and draw attention to *tobak*. The specific aims are to:

1. Review its history, with particular emphasis on its use in Africa and the Sudan. The study describes the history of its introduction to Sudan, and its botanical characteristics. The study also describes its use, its chemistry, locations and other properties. Methods of processing of *tobak* for consumption, social, religious aspects, production and marketing issues also are examined.

The report also examines data that pertain to trends in frequency of use of *tobak* and smoking of cigarettes, in the Sudanese population. Further research needs are considered. Since secondary sources on *tobak* are nearly non-existing, this study is almost based completely on primary sources, i.e., direct interviews.
2.2 Review of the Literature

2.2.1 Historical overview of tobacco and snuff

Snuff is a form of finely cut or powdered tobacco and flavoring agents. Snuff can also be defined as a pulverized form of tobacco that is either inhaled through nostrils (sniffed), chewed or placed between the gum and cheeks (Encyclopedia Americana). Habits related to the use of snuff and chewing tobacco differ in different parts of the world. The practices and terms used for these habits and customs also show worldwide variations. Descriptions of the terms, used and current practices of the habits in many countries and different areas of the same country is available (IARC 1998).

2.2.2 Origin of tobacco chewing

Tobacco was first used by the aboriginals living in South and North America (Curtin 1939). The origin of tobacco and related habits and customs is not precisely known. The topic has been reviewed recently (IARC 1995). It has been estimated that cultivation of tobacco probably dates back more than 10,000 years; the original use of the plant is probably smoking and oral consumption. The Spaniards in the late 16th century described the habit of oral chewing in parts of South America (IARC 1995).

Early writers differed on the origin of the uses of the plant tobacco. The possibility include several theories. The plant that Indians used for smoking the smoke of tobacco were baked clay, sand or natural rocks and were called the "Tobaga". It was reported that the Spaniards later slightly altered the name and applied it to the
The practices of tobacco habits and customs varied among native tribes. Chewing of tobacco mixed with lime or finely powdered burned shell seems to have been a common practice. The habit is practiced to give support against hunger, thirst, and fatigue (Curtis 1939). Chewing tobacco was also thought to have medicinal values, including cure of certain diseases, burn wounds, and relief of headache of the body and parts. Tobacco was used for the religious and pleasurable (Curtis 1939).
Powdered snuff and snuff with

Snuff was the first to be snuffed (snout tobacco) as an inhalation device, inhaled for its medicinal values, to induce smoking in revolting minds (Callin 1932). According to a relief who lived among the Mexicanos (1585-1999) Tobacco was placed in the mouth to add dizziness and stop effects (Christen 1982). Snuff was first constructed by the native Brazilian Indians (Callin 1932). Their practice spread to the spread of the 18th-19th century, inhalation of native tobacco.

Cured tobacco and tobacco with additives

For preparing snuff, tobacco, and vinegar were added tobacco leaves, leaves of the tropical tree, and then pulverized into the powder in a mold. Snuff and the essence produced were molded and shaped. Smoking was used from northwest American Indians (Callin 1932).

Spread in the world

The world was spread throughout the world (PACK 1932) and its own grid increased the grid. With the grid brought in with grid and grid with grids. The habit of the grid.

From Portugal. In 1566, Portugal
The history of mohair is closely tied to the vast expanses of the Mongolian steppe. The flocks of the domesticated Cashmere goat, a subspecies of the wild goat, were herded by the Mongol tribes, and the wool was used for making tents, clothing, and bedding.

Mohair fibers are also found in the wool of the Angora goat, which originates from Turkey. This goat was introduced to Europe in the 18th century and became popular for its fine, long, and lustrous wool.

In the 19th century, mohair production expanded significantly, particularly in the United States, where large flocks were established. The demand for mohair increased due to its high quality and durability, which makes it ideal for clothing and upholstery.

Today, mohair is produced in various countries around the world, including the United States, South Africa, and Australia. It is used in a wide range of products, from clothing to home decor.

In the middle of the 19th century, mohair was introduced into Russia and was used mainly in the upper class. It was first used only by the upper class and subsequently spread to the general Russian population. Since the turn of the 19th century, mohair has been used widely in Russia (ARC 1985).

When mohair was first introduced to Europe, it was only used in the upper class.
Beneficial effects of exercise were noted even contraband in the habit of using tobacco was widespread. To be sure, the 19th century King George V of England, a strong proponent of tobacco, decreased his own tobacco intake by 4,000 pints of beer to prevent the adverse effects of tobacco on Scotland. During the 1800s, the Emperor Wladimir Fedorovitsch prohibited the sale of tobacco and introduced physical and capital punishment for those who violated this law. Leaders such as Pope Pius VII and the leader of the Anti-Clerical League (founder of the Catholic church) also sought to limit its use.

1.7 Practice

Effects of:... and all differ in different social classes are affected and in decrease the health of an individual. Current practices have resulted in different approaches to tobacco use, resulting in higher morbidity and mortality by CIHC and WHO.
...
Kushari, a traditional Egyptian dish, is produced by boiling rice in water with salt, peeling, and soaking red lentils, green lentils, and red kidney beans. The mixture is then combined with a thick tomato sauce and served on a bed of rice. The dish is typically served in a clay bowl or a larger plate and is a staple in many Egyptian households.

In the Middle East, particularly in Egypt, Kushari is a popular street food, often found being sold by vendors on the streets, particularly during the holy month of Ramadan. The dish is also popular in other countries in the Middle East, such as Jordan, Lebanon, Syria, and Iraq.

Kushari is considered a comfort food and is a significant part of Egyptian cuisine, often enjoyed during special occasions and gatherings.
The habit of snuff taking in South Africa, was described by Reen (1955). While reporting on the etiology of respiratory tract cancer in the South African Bantu, Reen found that the habit was widespread among the older people and dates back to immemorial times. It is practiced for ceremonial, religious and medicinal purposes. In South Africa snuff is made from tobacco leaves and ash and occasionally ground oil or lemon is added; the product is inhaled through the nasal passages (Baaseman et al., 1971). However some tribes use it in the mouth (Higgings and Oettle 1980). Tobacco forms part of the snuff used by these tribes but is not the principle constituent; addictions of ashes, herbs of various plants and tobacco are often used. Among the twenty different plants in common use Aloe Marthili is the favourite plant. The plant is burned to ashes and subsequently ground with stones while small quantities of water are added until the substance becomes dark and slightly oily. Additives, as flavoring agents, like eucalyptus oil, lemon juice, and other aromatic herbs are occasionally used. Tobacco powder to form about two thirds is added to the plant mixture and subsequently ground. In different preparations, the proportions of the tobacco/ash mixture is adjusted according to taste. Snuff is either inhaled or used orally, historically inhalation seems to be the most common form of practice. The habit appears to be more common in rural than in urban areas. The habit is common among females than in males.

van Wyke et al (1985) described the oral habit among the Bantu of Africa. The snuff quid is placed between the lower lip and the gum. The saliva snuff mixture is repeatedly swallowed till it becomes bland. The nasal use of snuff in
rural areas of South Africa and Basutoland. (Powers 1971) is practiced by placing a pinch of snuff in the nostrils several times a day and even at night. The custom of snuff dipping in the mouth is found in urban areas. The constituents of the preparation used are not different from those previously described by Keen (1955)

van Wyke et al (1977) while assessing the oral health status of elderly colored South Africans, observed that they smoked cigarettes, zolle (a large homemade cigar), pipe, and have also chewed tobacco and dipped snuff. They also found that females indulged more commonly in tobacco chewing and snuff than smoking.

Kent studies used clinical data to obtain population estimates of tobacco usage. A few studies used surveys of healthy populations and obtained population estimates of this habit. Higginsam and Ollide (1980), using a survey of population of Johannesburg and rural hospitals estimated the habit of tobacco use among South African Bantu. From a sample of 1,144 individuals aged 15 or above, they found that 18% of males and 39% of females used snuff both orally and nasally. 21% of males and 37% of females in rural hospitals used snuff nasally. It was not clear how the Johannesburg sample was selected. Solomon (1975) studied a sample of 20,333 Swedes age 15 and older. 14.2% of males were found to be habitual users of tobacco and less than 1% of females. Salee et al (1984) estimated the use of snuff in Saudi population in a sample of 851 as part of health campaign. In an area Saudi Arabia, they found that 18.7 out of 500 were snuff users.
2.3 Materials and Methods

2.3.1 History and ecology of toombak plant

The source of data used in this study are interviews of persons living in Al-Yashir town, capital of Darfur region in the western part of the Sudan. The information was obtained by the author during a field trip to the town in January and February 1987. It is considered informal interviews to obtain data.

Al-Yashir town was established in the late 18th century (Encyclopedia Britannica). A historic caravan centre, it is located at an elevation of about 2,400 ft (70 m). The town serves as an agricultural marketing centre for Toombak, cereals and fruits grown in the vicinity of jabal (mountain) Marrah, 75 miles to its southwest. It is linked by road with Al-Jumaymah (a border town with neighboring Chad) and Usk Faddak.

Geographically, Darfur consists of large areas of rolling plains, approximately 170,000 square kilometers (660,000 square km) in area. The mountain, Marrah, has an average elevation of 9,200 feet (2,800 m), otherwise the plains of Darfur are relatively featureless, particularly in the north, where they merge into the desert. Soils, which are generally sandy or stony, support some seasonal grass and low, thorny shrub vegetation. The Marrah area receives heavier rainfall than other parts of Darfur, and a number of large wells (seasonal water sources) are rise in the mountains and filter southwards across the plains. These wells provide the fertile soil that is necessary for the growth of toombak.
Cultivation of *toombak* in Darfur was described by Mohamad (1969). *Toombak* is a semi-desert tobacco plant mainly confined to the Darfur region. The climate of Darfur, high temperatures, heavy rains during autumn, and presence of large wadis along the water courses draining the waterfall, provides variable soil conditions for production of *toombak*. The soils are of clay and loamy types, and the ground-water table is above the limit of root penetration. The tilled areas are preserved by the natives by a process of trial and error. In the past *toombak* was grown in limited areas, such as the silty flood plain terrace in the valleys of the Nadi Golo, around al-Fashir area, and its tributaries further south, especially at Kali and Maleba, but now it is widely cultivated in large areas of Darfur, extending to most Western and Southwestern areas of Darfur. The five main districts for *toombak* cultivation are: 1. Al-Fashir district; 2. Northern Darfur Darfur district; 3. Al-Gaz token district; 4. Western Darfur district (Zaling); 5. South Darfur district. The largest contribution is from Al-Fashir, in the Shagro-Tuwila-Shangbleh triangle Fig 1.

The plant is cultivated in its wild form. It is a dry season cash crop. The main cultivation areas are as shown in (Fig 1): Shagro, Golo, Tarlak, Yolki, Serif, Koba, Kora, Chite, Shingle lobbi, Serif Ome, Al-mollem, Al-Gaz token, Arara, Tabulet Garblo, Lumen Amin.

Almost all natives of Darfur are small scale farmers. It is estimated that about 90 per cent of *toombak* production in the Sudan is confined to Darfur. Al-Fashir is the main Centre for production and marketing of *toombak*. However, because smuggling is
Distribution for Tonkolik cultivation

TONKOLIK CULTIVATION AREAS IN DARFUR

NORTHERN

Sudan Area

Kukum

Kordofan

Central Africa

Bahr El Ghazal

10km

El Fasher

El Geneina

Golo

Gareisa

Ar Medhen
2.3.2 Processing and Curing

Data were collected by interviewing persons who are natives of Al-Fashir and knowledgeable about the history of toombak introduction to the Sudan, its farming, processing, and marketing. Among them were: persons involved in the retail and wholesale marketing of toombak, as well as members of Union of Toombak Sellers, toombak farmers, and the Union of Toombak Farmers. A small number of informants were selected for extensive interviewing because they were particularly knowledgeable about some aspects of toombak.

Toombak is an important cash crop in Al-Fashir, and hence interviewing persons aware of the profit made by farming and selling toombak even made them suspicious of the fact that the information could be used to change public opinion against the use of toombak. This is one limitation of the information obtained. Wherever possible the author referred to more than one source to clarify contradictory information. The evaluation of a source or informant became even more crucial when that source was the only source for certain facts. In an attempt to build up internal consistency, the information obtained was checked by bringing up the same topic more than once with all the interviewees.

2.3.3 Identification of the Species of toombak plant

For identification of the species of the native tobacco plant grown in Darfur, flowering whole tobacco plant samples were collected from various production areas in the region.
The samples were sent by the Faculty of Science, University of Khartoum, to Professor Ihab Al-Amin, an expert in plant taxonomy, who identified the species Abdul Al-bari (1988).

3.2.4 Definition and preparation of the

Both an electron and obtained from the Filer of the Ministry of Science, Administration and Energy, Research Authority at Khartoum. Fresh samples of these were collected from the areas of the mountain in Gezira. A sample of a certain sample to be known to contain radioactive elements, which are in a total, were obtained from the Gezira mountain in Gezira Region, in the Sudan. All samples were shipped to the lab, where they were studied for radioactive elements, and then were studied and also tested for their radioactivity.
2.4 Results

2.4.1 Historical aspects of toobak

Various stories were told about the history of the first introduction of toobak to the Sudan by many natives living in Al-Tushir town, and these stories were supported by interviews of persons knowledgeable about toobak.

According to a legend narrated consistently, toobak was first introduced by a Koranic (Islamic) teacher named Haji Wad Ameri. It is said that Wad Ameri was a Muslim Arab who came to the Sudan from Egypt before the reign of Al Imam Al-Mahdi (1868-1885), after traveling in West Africa. It was believed that he brought toobak seeds with him from Morocco. Wad Ameri subsequently settled in a region called Abusinade in Darfur. Wad Ameri, whose original mission was to teach the natives the Koran, also taught them how to grow toobak. Thus, the natives began to grow toobak plant for their own use as well as for commercial purposes.

Another story claims that toobak was introduced to the Sudan during the Ottoman era, and contemporaneously Turkey's colonization of the Sudan. A third story maintains it was also believed that the tobacco plant was introduced to the Sudan from Timbucto in West Africa.

2.4.2 The origin of the native name of toobak

It has been argued that toobak probably refers to its origin from Timbucto in West Africa. Al-saaf is a commercial name used in Darfur to describe the quality toobak plant. Other names include Wad Ameri, in reference to the person, Hussein Wad Ameri, who was believed to have
introduced it to Darfur. Sultan Al-kali, in another essay and it means, "the power to improve one's state of mind".

2.4.3 Species of tocshak

The flowering tocshak plant sample was investigated by Professor Isthaum, at the Department of Botany. The tocshak plant was found to be of species within the genus Nicotiana "Solanaceae," R. Rustica.

2.4.4 Cultivation of tocshak

It is said that when Hussein Nad Amari first introduced tocshak to Darfur the seeds were first grown in the area presently known, Shangle toshni, Kobbai and then shifted to Tawilla and gradually it became commercially produced in small quantities. The new cash crop attracted many natives, and accordingly the area was named "Shangle toshni talgi dabhni" which means "the area where, if you turn a brick you will find gold". Many natives moved to Shangle Toshni for "the new gold."

During the turn of the twentieth century tocshak became an important cash crop and major source of revenue. The consumption of tocshak increased over the years and it was exported to different parts of the Sudan.

Cultivation was largely expanded to include most of the North and Southwest of Darfur. The expansion in tocshak cultivation was at the expense of food crops, this lead the British Government (1938-1948) to impose high taxes for tocshak production and to apply new laws to restrict this production. It is told that, Al-Imam Al-Mahdi (1931-1955), the well known national religious Sudanese leader prevented
the use of *Loomah* as religious bases.

Despite the reputation of *Loomah* as the first cash crop, a significant expansion in its production took place only in the last twenty years, due to the following reasons:

1. During the British Colonial era (1899-1956) it was permitted to farm tobacco only after production of enough food crops; after the Sudan became independent in 1956, the implementation of the laws gradually became less effective.

2. Heavy taxes imposed by the British government on *Loomah* production, affected the sale profits and consequently, discouraged the natives from cultivating it. Recently, however, periods, the natives resorted to smuggling *Loomah* and make good profits.

3. *Loomah* was prohibited by Al-Iman Al-Mahdi, whose followers reside mainly in west of the Sudan. The belief in al-Mahdi faith was weakened over the years.

4. *Loomah* was regarded as a socially unacceptable habit by some tribes, because it was believed that it causes mental incapacities.

After the field is cleaned, the farmers make terraces of raised heaps of soil of about three feet high. This preparation is made in May before the rainfall. During the rainfall, the women and children cultivate food crops while the men would be busy in land irrigation in preparation for *Loomah* cultivation which is locally called "Tortura" a deep even textured loam.

The seeds are planted in October when rain ceases and the soil begins to dry. Sowing is done by mixing the seeds with sandy soil particles and then scatter them haphazardly on
The field surface covered by grass. Singing and weeding are done at the same time. Seedlings are planted in November when the surface is quite dry. Holes are therefore dug with the stick to a depth of about 6 inches, with a vertical face to the east (to protect from the sun). Individual plants are inserted in the deep slit at the base of the vertical face where, during the subsequent wilt, shade will protect the tender plant from the intense radiation of the morning sun (Photo 1).

During the period when Louzieh is growing, the farmer would be busy planting the "Magan" and foraging the perennial grass "Jenaj" until the plank flowers in about two months. This stage is called "Dunia" and the flowers are removed in three or four intervals for a better development of leaves; this stage is called "al-Noorir". Neither during transplantation nor afterwards during growth, is the plant irrigated. The plant attains maturity and stands 2.3 feet above the ground (Photo 2-3). By the time the leaves sicken they become yellow. The size of the leaves are smaller and are called locally "Daha al tarag", the next stage is also called again "Dunia".

Leaves are harvested in special baskets called "Kafayat" (Photo 4). The plant's main stem and branches, other leaves were harvested and called "Kulo".

Harvesting of tobacco leaves begins in early December and continues to March (winter season). Tobacco leaves are harvested when they turn yellow and brown spots appear on them and this stage of harvesting is called "al-goada" which means small pox stage. The harvested leaves are
1 Planting toombak.

2 Toombak plant flowing.
3 Toombak plant field.

4 Toombak plant carried with cutayat
attached in small baskets, which are then emptied on the
ground in a naturally ventilated shade (Photo 5). Each heap
is left on the ground in the shade for three days during
which time the leaves are turned over occasionally to
achieve uniform drying. The color starts to change from
yellow to brown and dark red. Subsequently, they are tied
in bundles and moistened by sprinkling water. The bundles
are stacked for fermentation for a couple of weeks then
prevented and dried again. All the small heaps are gathered
in large collections each composed of about twenty basketfuls
of toombak, (Sabra; Photo 5). The temperature is usually
72 to 75°C. The toombak leaves in the "sabra" are
continuously over turned for at least three months until
they dry up. The "sabra" is a very important fermentation
stage, the drier toombak leaves become, while in sabra, the
barker would be their quality and consequently price would
be higher.

After sabra, toombak is aged for one to two years by
storage. During this aging process natural fermentation
occurs. Although all parts of the toombak plant can be used for
confection, the leaves make the best quality. However,
meantime for commercial reasons, to increase the quantity
and weight of toombak, flowers and stems are added to
the toombak leaves. Furthermore, factors such as the soil,
climate, methods of fermentation and curing also determine
the quality of toombak.

Toombak is graded according to the quantity of the
leaves. The more leaves it contains, the higher the grade.
It is generally graded into three grades. The natives ter
the quality of tobacco by its color, taste, and smell; they also consider the type of soil in which it was cultivated.

The dry tobacco is transported to tobacco mills in Al Fakher, after storage for at least a year. It would be ground to a coarse powder, which would be used as a final tobacco product to be processed with tobacco for consumption. Powdery tobacco may be stored for many years. The tobacco products are exported in large bags (Photo 6) to different parts of the Sudan and some neighboring countries, Chad, Saudi Arabia, and Uganda.

2.4. Atrion

Description and Chemistry

Atrion is a sodium borate mineral and is called "Atrion" in Arabic. Atrion is also called "Seria" and has the chemical formula Na3B10(OH)2(BO3)2.2H2O. It is mainly used in the tobacco industry. It is also used in glass-making industries.

Many wells and boreholes show a fairly high concentration of boronates and borax in ground water, especially in the west and north-west of the Sudan.

Atrion is found in Wadi al atron, Khossib Lake, Makhila, Makhila and crater lake in the North and North-west of the Sudan. Analysis of atrion from the above sources are shown (Table 1).

The results of geochemical analysis of atrion for trace elements are shown in (Table 2). Atrion samples tested for radioactivity by autoradiography showed no appreciable trace elements enrichments in any of the analyses undertaken compared with control 2 and 3 (Fig.5).
Table 1: Chemistry of Alcor obtained from different sources

<table>
<thead>
<tr>
<th></th>
<th>Mallorca crater Lake</th>
<th>Lake Nud</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na2CO3</td>
<td>52.7%</td>
<td>17.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>33.5%</td>
<td>8.0%</td>
</tr>
<tr>
<td>Organic Matter</td>
<td>5.5%</td>
<td>14.7%</td>
</tr>
<tr>
<td>Undetermined</td>
<td>8.3%</td>
<td>22.3%</td>
</tr>
<tr>
<td>Moisture</td>
<td>39.2%</td>
<td>39.2%</td>
</tr>
</tbody>
</table>

No nitrate or borate is found in either sample.
### Table 2: Elemental Contents of Atron

<table>
<thead>
<tr>
<th>Element</th>
<th>Sample NO 324 24%</th>
<th>NO 32-25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>11.9</td>
<td>0.5%</td>
</tr>
<tr>
<td>Mg</td>
<td>0.022</td>
<td>0.0%</td>
</tr>
<tr>
<td>CO3total</td>
<td>10.3</td>
<td>11.6</td>
</tr>
<tr>
<td>HON3</td>
<td>5.2a</td>
<td>4.67</td>
</tr>
<tr>
<td>Cl</td>
<td>9.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Na</td>
<td>13.2</td>
<td>12.2</td>
</tr>
<tr>
<td>Element</td>
<td>(Units)</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>30.30</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>32.12</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>26.00</td>
<td></td>
</tr>
<tr>
<td>Rb</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>43.29</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>106.09</td>
<td></td>
</tr>
<tr>
<td>Cs</td>
<td>19.00</td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>9.00</td>
<td></td>
</tr>
<tr>
<td>La</td>
<td>5.00</td>
<td></td>
</tr>
<tr>
<td>Eu</td>
<td>42.00</td>
<td></td>
</tr>
<tr>
<td>Tb</td>
<td>7.00</td>
<td></td>
</tr>
<tr>
<td>Yb</td>
<td>9.00</td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Ta</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>
Control shows positive radioactivity

Atron shows negative radioactivity
The preparation of khat or qat involves soaking the dried leaves in water and mixing the resulting paste to precipitate forms. The precipitate is then removed and tobacco powder added to the solution and mixed until the substance is moist and sticky. The prepared khat is kept in airtight tins for a few hours before it is used for consumption. The quality of the final product depends on the quality, taste, and price, and its concentration affects. The composition of the commercial preparations are generally similar with very little difference due to the experience of the individuals making the preparation and the variety of khat used, depending mainly on its leaf and specific cultivation. Khat is grown in Yemen in a combination of heat and quality. While the methods of preparation and practices of khat in South Arabia are essentially similar to those of khat in other regions, incorporating it may be added to khat at the discretion of the user (Elston et al. 1993).

The leaves used for consumption in the called khat in the homes include South and Yemen. The plants used in Yemen are known as khat or qat. The leaves that are used are the ones that can be used to make a paste. These leaves are placed in the top of the chewed khat. This chewed khat is then placed in the mouth and chewed. This chewed khat is then placed in the mouth and chewed.
in the east, west, and in the southern parts of the Sudan i.e. Northern, Eastern, Central, and western (Kordofan), Western (Darfur). About 80% of the Sudanese population reside in these areas. Published work about the psychology of addiction of the Sudanese to the habit of khat (toobah) taking is lacking. It appears that usage is an associated behaviour generally from the surrounding society and friends. The habit of khat taking begins in early adulthood, with very little exceptions of individuals with the habit in earlier life. The rationale for initiation to the habit is usually its medicinal value, such as alleviation of toothache, or permission from a friend addicted to the habit or, occasionally, may be from peer pressure. The habit of use of khat seems to be an acquired taste. The first quid (matta) causes a highness and urgency as toobah has a stringent taste and a pungent smell. However, repeated experiences, three or more would expose the user to the alkaloid, nicotine, the principal factor that creates continuation and addiction.

Toobah is sold in the local market by toobah sellers. (Photo 4) keep it in large air tight, tin called hookah. Toobah is sold to the consumers in small plastic bags (Photo 3) or in toobah pocket containers also called of hookah. The bag or hookah contain several quids or (mattas) as they are locally called. A hookah or bag full of toobah usually lasts the average man a day. The mix- the quids and frequency of usage vary between different individuals.

Use of toobah is a poor person’s luxury, as the cost of a hookah or plastic bag full of toobah is less than one
8 Toombak processing.

9 Toombak ready for sale.
10 Toombak sellers

11 Toombak quid (suffa)
12 Sulla in the lower lip.

13 Squamous cell carcinoma in the lower lip in hookah user.
Length of cigarette package of 10. Despite the fact that toobak is generally less acceptable than smoking, many toobak users were originally smokers who switched to use of toobak because of high cost of cigarettes.

The method of use of toobak (Photo 11 13) is as follows. The habit is usually practiced by taking a small portion of toobak, placing it in the palm, usually of the left hand, and manipulating it by the fingers of the right hand until it forms a Glob. Then the glob is placed in the gingival alveolar or buccogingival groove for a period ranging from few minutes to several hours until it becomes bland.

There are differences in males and females regarding the sites of insertion of the naffa. The females tend to use sites that are not visible when they place naffa in the mouth because socially it is more acceptable for females to be seen than males. The usual common sites for insertion of the naffa in males are the vestibule of the lower lip, lower buccal vestibule, upper labial and lower buccal vestibule and sublingual anterior and posterior toward the lateral side. For females, the most common sites for insertion of the naffa are the lower buccal and upper vestibule and sublingual anterior and posterior toward lateral side and lower lip. Periodically the user eats the buccal debris that is freed from the buccal and the naffa which is secreted during toobak use. The mouth is usually rinsed with water after the naffa is removed. The process is repeated several times most of the waking hours. The naffa is sometimes retained in the mouth during sleep. The ashtray of one are uniform throughout the salon.
Te responded during the day was the holy month of Ramadan (the Muslim fasting month). From early dawn to dusk, the nifta also lasts to be removed during prayer times, which are five times a day every day of the week.

2.3 Production and Economics

Stories were told that the use of teesbak spread very quickly throughout Darfur and other parts of the Sudan soon after its introduction.

Al Fashir is the main centre for production and marketing of teesbak and serves as an indicator for population consumption. In the years 1983-84, production of teesbak was exceptionally low. However, between 1985-1987, the production of teesbak increased significantly. This increase was due to the following factors:

1. Teesbak is not usually affected by drought
2. Rainfall of low yield from good areas that grew shifted to teesbak.
3. Displacement of large numbers of people to teesbak production areas by effects of drought.
4. High cost of cigarettes

The interviews reported that teesbak areas have expanded over the last decade. Most of the expansion took place in H., West and southeastern regions of Darfur. The most critical areas are the area around Al Fashir province.

Tuba, Tarna and Shaga (Fig 1) it was estimated that annual production of Tarna, Tuba, Sharga, Tushai and Shaga are follows: 600, 1,100, 3,000, & 800 long respectively. The production is very low in Eastern Darfur. The new areas include, Nubia, Umara, Lumana, Kufra. The old production
areas were, Shangil Tobel, Kao, Zalinga. These areas produce about 40% of all Darfur production of konsohak.

The informers believe that the reasons for the increased production were skyrocketing prices, which caused farmers to become sellers and sellers to become producers. The increase in price of cigarettes was another factor which caused many people to switch to use of konsohak. Also markets were greatly expanded in new areas of the Sudan. It has been said that large quantities are smuggled to Saudi Arabia, Egypt, Yemen, Libya, Ethiopia, Zaire, and Chad.

The pricing of konsohak is established by a joint committee of sellers, and local administrative officials. The main markets are in Al Fashir, Nyala, Zalinga.

2.4.9 Use of konsohak and religion

The use of konsohak soon became a controversial issue. Islam religion requires a Muslim to maintain a clear mind especially during prayer. Soon the native became aware that konsohak clouds the mental abilities of the individual and religious Muslins began to become skeptical about the use of konsohak. They formed a group of learned persons to seek the formal religious opinion about its use. Wad Amai was considered the most suitable individual to consult, since he was a Koranic scholar as well an introducer of the konsohak plant to the area. The group of religion went arrived at Wad Amai's village at about sunset. They told Wad Amai about the purpose of their visit. Wad Amai promised to respond to their queries the following morning.
Wad Amari took them to a place that he had prepared for them to spend the night. He brought a donkey with him to the place and told them this donkey was for the evening meal and then he left. He came early next morning, found the donkey lying in the same place, and asked them whether they had eaten anything. They said, "how can we eat a donkey? everyone knows that eating donkey's meat is forbidden by religious law". He told them that he did not mean that they should eat the donkey but he meant that they could sell the donkey and buy food. Then he told them that this given the answer to their queries about the formal opinion of religion on use of taoufak : "One can sell it but one should not eat it". By asking this statement Wad Amari justified the production of taoufak by Muslims. Taoufak production took its rise in Darfur from this event.

Al-Tazak Al Mahdi the Sudanese Islamic nationalist leader, banished its use altogether among his followers, who mostly lived in this region. During the Anglo-Egyptian colonial era, the British government imposed heavy taxes on taoufak to discourage its production at the expense of food crops (Abdul-Baet 1987; Hamzoud 1997).

Taoufak survived all these restrictions and the habit spread all over the Sudan. The production was further encouraged by the private enterprise, since substantial profits were to be made by farming and selling taoufak.
2.5 Discussion

This study identified the species of the native tobacco grown in the Sudan for manufacture of narghile within the genus Nicotiana (Solanaceae). Nicotiana rustica. Mexico was believed to be the original land of N. rustica (IARC 1989). Although the vast majority of smokeless tobacco products are used in North America and western Europe, N. rustica is used in tobacco containing chewed products in USSR, Iran, Afghanistan and some parts of India (IARC 1989).

The information obtained on its history in the Sudan by interviews has inherent limitations. Oral histories are affected by the level of education of the informant, openness, and knowledge about the subject of interview. For about three hundred years narghile has played an important role in the life of the Sudanese people and hence the neglect of historical treatment is surprising. A special effort should be made to document the history of this substance.

There is a strong belief that introduction of narghile or narghile is attributed to Nad Awari who is thought to have introduced it from Timbuktu. Alternative views include introduction of narghile from Turkey during the Ottoman era.

In the absence of documentary evidence to support these views, it is important to consider the historical events occurring at that time. Reports indicate that in the sixteenth century, on the West coast of Africa tobacco became a commercial product (Axton 1975). Timbuktu was then a thriving trade centre in that part of Africa (Encyclopedia Britannica). Timbuktu, also spelled Tombucto, a city in
the West African nation of Mali, is located on the southern edge of the Sahara, about 10 miles (16 km) north of the Niger River. It was founded about AD 1100 as a seasonal camp by Tuareg nomads. Historically, Tinuku, was an important post on the Trans-Saharan caravan route and as a centre of Islamic culture (A.C. 1400-1600). It was also an important focal point of gold-salt trade. With the influx of North African merchants came the settlement of Muslim scholars. The city scholars many of whom had studied in Egypt or Nokkha in Arabia Peninsula attracted students from a wide area. It is likely that Mal Amari, being an Arab and Kuranic teacher possibly of Egyptian nationality or from Arabia, travelled to West Africa as a part of the early Islamic missions, and from there he travelled to the west of the Sudan. Review of the history of Darfur and its link with prehistoric states in the west Africa, would provide further support to the introduction of Kounchak from west Africa. The history of the Trans-Saharan Islam which had linked Arabians, Northwest Africans and the Sudanic Muslims suggests that Darfur was a part of the Trans-Sahara caravans. By the early 15th century; the powerful state of Sheriffs descendants was established in Forno (Maghrib). Meanwhile a number of small states were strong from one end of the prehistoric Sudanic region to the other, (Senegambia, Songhai, Air, Mossi, Hoh, Tama, Koro -Borum, Darfur and Tanj). The latter two are parts of the present the Sudan.

Islam was introduced to these areas along trade and pilgrimage routes through the efforts of a number of learned families. The Muslim states of the Sudanic belt were in contact through Trans-Sudanic pilgrimage, with each other and with Maghrib, and Egypt.
The alternative view of introduction of _toombak_ from Turkey or Arabia is also likely as tobacco arrived in Turkey in 1505. _Kabaholak_ is a Turkish tobacco sometimes used in pipe tobacco blends (Weber 1985). In the Sudan Tobacco used in water-pipe is called _kobakeh_ or _geesheh_.

There is a similarity in the native names used for tobacco in the Sudan and Persia. The name used in the Sudan is also used Persia (Weber 1985), and may have been introduced to Arabia as historically there have been strong cultural relationships between Persia and Arabia. Tobacco reached Arabia in 1063 (IARC 1985). _Toombak_ in Persia is a term of tobacco smoked in a deep state in the nargile or _geesheh_. Live coals are placed on the top of the _toombak_ to light it; (Weber 1985).

It was not known why the natives shifted from the nasal use of _toombak_ to the oral use. _Toombak_ is probably more of a source of pleasure to taste than smell and more effective at the same time in production of dependence when used orally.

Interview of individuals living in Al-Fashir town could not provide useful information neither on the history of _toombak_ as an additive to _toombak_. Arson, supposed to exist in other parts of the world, has probably been added to _toombak_ for its alkaline effects. It has been shown that at high pH (11.0–11.8), nicotine is completely protonated and its rate of absorption is thus accelerated (Brunnmann, 1974). It was found that many samples from the USSR, which contain lye, have remarkably
high pH (11-11.8). When placed in the mouth nicotine reaches the central nervous system very quickly. The pH of tobacco-containing products influences the absorption and thereby the extent of pharmacological activity of nicotine (Bresnahan et al 1985). Atron was used as an additive possibly because it accelerates absorption of nicotine, and increases its action on the central nervous system.

Data on Toombs suggested that production increased significantly since 1985. Furthermore, with increase in prices and concomitant restrictions on import of cigarettes in the later years, there is a tendency to switch from smoking of cigarettes to use of toombs.
Chapter Three
Descriptive Epidemiology
3.1-Introduction

The main objective of this study is to report on the relative frequency of oral neoplasms in the Sudan and their association with the use of Youmbak (local snuff). The incidence of oral neoplasms on a global scale has been reported by Waterhouse, (1976). Only limited data from the developing world were reported. This study examines the relative frequency of oral neoplasms (WHO 1976) within the Sudan and compares it with other countries. The best available means was the use of published tumour registration data. The specific aims were to determine the relative frequency of oral neoplasms as a proportion of all neoplasms at all body sites, and to analyse the data for differences in age, sex, ethnic groups, and/or geographical distribution, use of Youmbak.
3.2. Review of the Literature.

3.2.1 History of cancer in Africa

Although the incidence and pattern of cancer has always been of interest to cancer epidemiologists, the data from Africa are both incomplete and less recent. Early reports stated that malignant disease and epithelial tumors in particular were extremely rare in African countries, while lice tumors are more common than elsewhere (Hoffman quoted by Limet, 1962). However, evidence for cancer in Africans is available as early as 1881 in the records of Kabwe Hospital in Kasempa. Analysis of data recorded in this hospital in the period 1937–1956 showed that all types of cancer occurred in Africans; cancer of the cervix, penis, skin, and liver were the most common forms. Other early reports also provided evidence on occurrence of cancer in Africans (Stoeken, 1934; Vint, 1935; Smith and Elms, 1935).

The argument of rarity of cancer in Africans has been widely discussed and proved to be false (Wills, 1968; Hoffman, 1962). This is only because the argument is based on comparisons of occurrence of cancer in African populations with that from the western world, with very little consideration for the differences in standards of health care in the two communities. Smith (1970) stated that "Comparison between the incidence of a disease in different places and different times is always difficult, particularly if the disease is one that requires a high standard of medical care before it can be diagnosed and which varies sharply in incidence with age."

Cancer incidence is known to vary with age and tendency for increased morbidity and mortality is high in an older
age group. Therefore, any comparison of cancer incidence made between two communities with different age structures should examine such differences before any conclusions are offered. The young age structure of African populations should be considered. In developed countries, mortality occurs primarily in persons of sixty-five years or older, while in developing African communities most people tend to die before this age (Davies, 1969).

Incidence data on cancer was not available in Africa until the 1950’s. The first population-based cancer registry was established in Kampala in 1951 (Davies). The Kampala cancer registry operated for the people of Suasa Kyanudo, the region including Kampala and its environment. Davies (1961) reported the first incidence data from Kampala Cancer Registry (1951-1959). The region of Kyanudo comprised a population of just over 200,000 people in 1959. This survey coupled with a 20 per cent sample census enabled the first crude incidence rates to be calculated.

Using the results of this survey, Davies was able to compare the incidence of cancer by site and type with incidence rates in other countries. There was a steady rise in the cancer incidence rates of Africans to the age of about 40-45, but after this age the incidence tended to fall. In other countries it rises but it has been concluded that this fall in the cancer incidence rate after the age of 45 is a real biological phenomenon of cancer in Africans. We observed a very different cancer pattern from that which existed elsewhere. We observed that the two major cancers in Europe, lung and gastrointestinal, particularly colon
cancer, were less frequent in Uganda while penis cancer, primary liver cancer, skin tumors especially Kaposi's sarcoma and lymphomatous tumors were more common.

At about the same period, incidence rate survey was reported by Gottle and Higgenson (1960) for the South African Bantu and coloured races in Transvaal.

In 1962 using age specific incidence rate, Knovelden was able to report a comparative study of cancer occurring in African and Western populations based on the population rates for Kyadondo, Uganda from census in 1948 and 1956 and cases investigated over the period 1961-1962 and comparison with Norway 1953-1958 (Pederson, 1961), the United States 1947-1949 (Dorn and Cullen, 1959) and Johannesburg (Higgenson and Ultez, 1960). The result were similar to earlier studies by Knovelden (1961). The incidence of various types of malignant disease was much less in Uganda than Norway or the United States. The age specific incidence rates for both sexes in African and Western populations were similar up to the age of 49 years. Again the steep decline towards the old age group was observed.

In a similar study, Minton and Higdon (1963) compared the incidence of four of the most common cancers in Ibadan with those in the United States whites and non whites.
3.2.2: Relative frequency of oral cancer in Africa

Throughout previous decades reports on cancer in Africa indicated that oral cancer was low among Africans. Hickey (1958) investigated 1337 malignant epithelial tumors in the Sudan, and found that 130 were tumors of the mouth. The relative frequency of various oral tumors were as follows:

- Tumors of the salivary glands (pleomorphic adenomas) 58 (4.2%); mouth, including lips, 51 (3.8%); tongue, 7 (0.5%), and melanoblastomas, 16 (1.2%)

Davies (1961) provided useful information on oral cancer in Uganda. Using the crude incidence rates obtained from Kyadondo registration, he found that the percentage of oral cancer (ICD 149.5) out of all malignancies was 0.82% compared to 9.2% in London. He further drew attention to the high rates of jaw tumors in African children in Uganda. He observed that about 50% of all malignant tumors in African children in Uganda were lymphosarcomas, and 50% of those tumors occur in the maxilla and mandible. He further stated that this common childhood tumor also affects the salivary glands and other tissues.

Linell and Hartyn (1980), using the register of biopsy specimens from patients examined at Medical Research Laboratory in Nairobi, Kenya, from 1957-1981 examined a total of 2,747 records. They provided the relative frequency of various types of malignancies in Kenya, and compared their findings with those from French West Africa, Ghana, Nigeria, using a WHO report prepared by Edington (1961). The relative frequency of oral tumors including
Linsell (1967) studied the relative frequencies of various tumors recorded by the tumor registry at Nairobi, Kenya during the period 1947-1963 and compared his findings with its neighbors, Tanganeka (now part of Tanzania) and Uganda, using approximately the same number of tumors collected over the same period. A low frequency of oral cancer was reported from the three countries, as shown in Table 8. The high frequency of mouth cancers in Tanganeka is due to inclusion of Indian patients who were removed from studies of Kenya and Uganda.

Nina (1978) provided the first report of the distribution of malignant disease in Tanganeka from case series of autopsy and biopsy material (1969-1973) in Dar es Salaam. The relative frequency of mouth cancer as percentages of total cancers of the alimentary system over the period was as follows: 1969 (23.32%), 1970 (23.23%), 1971 (21.32%), 1972 (25%), 1973 (25.4%), and 20.9% out of the total number of cancers of the alimentary system. Cancer of the mouth showed a high concentration around areas with high density of hospitals or within 10 kilometers from a hospital.

Prabha et al. (1980) studied the relative frequency of oral cancer in Kenya, from 1968-1970. They concluded that oral cancer was low in Kenya accounting for about 2% of all malignancies. The highest rates in Africans were found in Kenyans of Somali origin and in Akikuyu. However, the highest rates in Kenya were observed in Kenyans of Asian origin. Cancer of the tongue and palate were the most common.
In middle Africa, using crude estimates based on relative frequency data cancer of the liver and lymphoma were the most frequent. In West Africa, they observed high rates of liver cancers and of lymphoma were observed in young individuals.

They observed different patterns of cancer in North Africa. Rates of gastrointestinal cancers were low, while breast cancer exceeded cervical. Rates of bladder cancer were high in Egypt, and the highest rates of oral and pharyngeal cancers in North Africa were reported from Sudan and Algeria.

Parkin and Muir (1984) provided an estimate of the worldwide frequency of twelve major cancers. They examined the incidence, mortality and relative frequency data and estimated the number of cancer cases in twelve common sites of all cancers that occurred in 1975 in the 24 areas for which WHO published population data. In males, cancers of the mouth/pharynx were the fourth most common cancers. They ranked behind cancers of the lung, stomach, and colon/rectum. In females, cancers of the mouth/pharynx were the sixth most common ranking behind cancers of the breast, cervix uteri, stomach, colon/rectum, and lung. Using population based data from several centers in East Africa, as well as relative frequency data, he concluded that esophageal cancer is more frequent than stomach cancer, and liver cancer is very frequent. In females, cervical cancer was twice as frequent as breast cancer.
In the Sudan, a COO report (Kuza 1980), using data from the Sudan Cancer Registry (SCR), 1968-1977, and the Radiotherapy and Isotope Center Khashm el Girba (RICK), 1976-1980, estimated the relative frequency of cancer at all body sites from each source. The relative frequency of oral cancer (ICD 140-145) from SCR data, was 4.1%. The figures for various intra-oral sites were: lips 0.66%; tongue 0.76%; salivary glands 0.85%; mouth (ICD 143-5) 1.96%. Cancer of all oral cavity sites combined (ICD 140-5) accounted for 3.4% of female cancers and 4.8% of male cancers. The frequencies of oral cancer from RICK data were: salivary gland tumors, 1.1%; oral cavity carcinomas (including tongue) 7.5%, and lip carcinomas, 0.8%. The relative frequencies of oral cancer sites combined was 7% in females and 12.9% in males.

3.2.2. Age-standardized rates for oral cancer

Each successive volume of the WHO monograph series, Cancer Incidence in Five Continents, included data from many parts of the world, the only exception being Africa. Because of failure of African countries to keep up their cancer registries with the WHO standard, the number of African countries contributing data has decreased from four countries in Vol. I and II to one contributing country in Vol. IV, while Vol. V did not include any data from Africa.

The most extensive compilation of data on cancer from developing countries is available in WHO, Cancer Incidence in Developing Countries (Parkin, et al 1992). Using age-standardized cancer ratios, crude rates and standard rates of cancer sites, and ICD International Classification of
Diseases nomenclature this monograph presented data from 87 cancer registries in developing countries and data from a register confined to developing populations in developed countries i.e. Register in USA. The report included data from 21 Centres of Cancer Registrations in Africa in 18 countries.

Table 4 shows cancer registries in Africa that have produced sufficiently reliable data to be published in the WHO monograph. (Parkin et al 1988). For each of these areas data on age-standardized oral cancer ratios (ASCR) for tongue and mouth cancer (ICD 140-145), were extracted. The presentation of data for individual oral subsites made possible the examination of the distribution of oral cancer according to intraoral subsites in Africa (Table 5). It would also be useful to study from the figures provided the comparative epidemiology of oral cancer grouped at one category.

The highest ASCR of oral cancer in males were reported from the Sudan, Rwanda, Tunisia, Gabon and Madagascar FIA 2. Females ASCR were generally lower than males. No population based data are available in these countries. The figures are based on relative frequency ratios which have been used to estimate site-specific rates for those countries, using a hypothetical “all sites” incidence of 100 per 100,000 (males) and 100 per 100,000 (females).

In Sudan, oral cancer was the second most common, ranking after cancers of the nasopharynx in males. Among oral cancers, cancer of the buccal cavity (143-5) is by far the most common followed by cancer of the lip (140.8) and
<table>
<thead>
<tr>
<th>Country</th>
<th>Institute/Location</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algeria</td>
<td>Histopath., Algiers, Orno 2</td>
<td>1966-1975</td>
</tr>
<tr>
<td></td>
<td>Constantine</td>
<td></td>
</tr>
<tr>
<td>Angola</td>
<td>Dept. Path., Univ. Hospital</td>
<td>1977-1979</td>
</tr>
<tr>
<td>Egypt</td>
<td>Cairo Metropolitan Cancer Registry</td>
<td>1973-1984</td>
</tr>
<tr>
<td>Kenya</td>
<td>National Cancer Registry</td>
<td>1963-1978</td>
</tr>
<tr>
<td>Kenya</td>
<td>Dept. Path., Coast General Hospital, Mombasa</td>
<td>1951-</td>
</tr>
<tr>
<td>Liberia</td>
<td>Liberia Cancer Registry</td>
<td>1976-1980</td>
</tr>
<tr>
<td>Madagascar</td>
<td>Anatom Path. Lab., Institut Pasteur</td>
<td>1979-1981</td>
</tr>
<tr>
<td>Malawi</td>
<td>Register of Tumor Path</td>
<td>1976-1980</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Ibadan Cancer Registry</td>
<td>1976-1979</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Zaria Cancer Registry</td>
<td>1976-1990</td>
</tr>
<tr>
<td>Rwanda</td>
<td>Dept. Anatom Path., National Univ., Butare</td>
<td>1970-</td>
</tr>
<tr>
<td>The Sudan</td>
<td>The Sudan Cancer Registry</td>
<td>1973-</td>
</tr>
<tr>
<td>Sudan</td>
<td>The Radiation &amp; Isotope Centre Khartoum</td>
<td>1967-1984</td>
</tr>
<tr>
<td>Swaziland</td>
<td>Swaziland Cancer Registry</td>
<td>1970-1983</td>
</tr>
<tr>
<td>Tunisia</td>
<td>Institut Salah Aliz, Tunis</td>
<td>1978-1980</td>
</tr>
<tr>
<td>Uganda</td>
<td>Kampala Cancer Registry</td>
<td>1978-1980</td>
</tr>
<tr>
<td></td>
<td>Entebbe Hospital, West Nile District</td>
<td>1981-1983</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Tanzania Cancer Registry</td>
<td>1980-1981</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Kilimanjaro Cancer registry</td>
<td>1979-1982</td>
</tr>
<tr>
<td>Zambia</td>
<td>The Cancer Registry</td>
<td>1981-1983</td>
</tr>
<tr>
<td></td>
<td>Of Zambia</td>
<td></td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>Dept. of Path., Ndola Central Hospital</td>
<td>1978-1977</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>Bulawayo Cancer Registry</td>
<td>1973-1977</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>Central Hospital Laboratory, Harare</td>
<td>1981-1982</td>
</tr>
</tbody>
</table>
Fig. 2 Age Standardized Cancer Rate
Per 100,000
For Males Africa
<table>
<thead>
<tr>
<th>Country</th>
<th>LIP</th>
<th>TONGUE. SALLIVARY MOUTH</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angola</td>
<td>0.5</td>
<td>1.9</td>
<td>7.2</td>
</tr>
<tr>
<td>Gabon</td>
<td>0.0</td>
<td>1.8</td>
<td>9.5</td>
</tr>
<tr>
<td>Kenya Regional</td>
<td>0.3</td>
<td>1.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Kenya South African</td>
<td>0.9</td>
<td>2.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Liberia</td>
<td>0.2</td>
<td>1.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Madagascar</td>
<td>0.3</td>
<td>2.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Malawi</td>
<td>0.7</td>
<td>0.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Nigeria (North)</td>
<td>0.5</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Nigeria (South)</td>
<td>1.1</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Nigeria (West)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Pakistan</td>
<td>0.0</td>
<td>3.3</td>
<td>6.4</td>
</tr>
<tr>
<td>Sudan (NCR)</td>
<td>0.9</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Sudan (RRR)</td>
<td>1.9</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>South Africa</td>
<td>0.6</td>
<td>0.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Tunisia</td>
<td>4.4</td>
<td>2.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Uganda</td>
<td>0.4</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Uganda-Kenya</td>
<td>0.4</td>
<td>3.8</td>
<td>2.0</td>
</tr>
<tr>
<td>U. K. of Tanzania</td>
<td>0.5</td>
<td>1.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Tanzania</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Tanzania Elburgo</td>
<td>0.4</td>
<td>0.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Zambia</td>
<td>0.0</td>
<td>2.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Zambia-Niello</td>
<td>0.8</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Zimbabwe-Rhodes</td>
<td>0.1</td>
<td>0.6</td>
<td>2.1</td>
</tr>
</tbody>
</table>
The relative frequency of oral cancer (ICD-10 140-5) is high in Burundi. It is the third most common type of cancer, ranking after cancer of the lung and larynx. Among the intraoral sites, cancer of the buccal cavity was the most common followed by cancer of the lip.

Most neoplasms were the second most common after the neoplasms of connective tissues in Burundi. The relative frequencies of oral neoplasms were as follows: buccal cancer, 3.4%; tongue 3.4% and salivary gland neoplasms, 2.7%. Cancer of the lip was extremely rare (0.0%).

In Burundi, oral cancer was the third most common after bronchial and Non-Hodgkin lymphoma. Cancer of the buccal cavity 7.8% (ICD-0 143.8-145.8) was most common; and cancer of the lip (0.0%) was extremely rare.

In Kigogome, oral cancer was the third most common after connective tissue and skin cancers. Cancer of the buccal cavity (ICD-0 143.8-145.8) was the most common; and cancer of the lip was rare.

It is clear that the data on ASCA presented above indicate that oral cancers grouped as one category (140-145) are very frequent in some African countries, and they rank as the second or third most common neoplasms in groups with overall increase in incidence of oral cancer. A consistent finding was that for the intraoral cancers, buccal cancer was the most common. Second, cancer of the lip was very
rare in these areas, the only exception being Tunisia where lip cancers were not uncommon. Age-standardized rates from other parts of developing countries also suggest that oral cancer is even more frequent. Table 6 shows a high ASR in many countries in Asia. Again, tongue and buccal cancers were the most frequent.

3.2.4 Incidence of oral cancer

Data on incidence of oral cancer for the developed world is available from the WHO monograph series *Cancer Incidence in Five Continents* (Doll et al., 1968; Doll et al., 1970; Waterhouse et al., 1976; Waterhouse et al., 1982). However, only little information is available for developing countries. The International Classification of Diseases (ICD-0) (World Health Organization, 1977) classified oral cancer in the group comprising numbers (140-149): lip rubric 140; tongue rubric 141; mouth rubric 143-145; and pharynx 148-149.

Pindborg (1980a), remarked on the confusion as to the precise anatomical location of cancer of the lip. Cancers of the lip consist of those arising from the vermilion border and the labial mucosa. In the International Classification of Disease for Oncology (ICD-0) (World Health Organization, 1977a) which forms the basis for the topography in the ninth revision of the ICD (1977), the vermilion border and the labial mucosa are separated. The terminology also defined tumors which extended over other anatomical structures. Such separation is important because tobacco related oral cancer affects the mucosal aspects of the lips.
3.2.5-Descriptive studies on the association between use of smokeless tobacco and oral cancer

3.2.8A Asia

As early as 1905 Pear pointed to the high frequency of cancer of the buccal cavity in India. He published notes on 377 cases of epithelial cancers seen in two years in South India; 91.4% of these were in the buccal cavity. In the same year Bentall reported analyses of 1700 cases of oral cancer in South India; 70.6% were cancers of the buccal cavity (including that of lip, tongue, and jaw).

Abbe (1915) found that 35 out of 100 mouth cancer patients were smokers, 13 chewed tobacco, and 1 used snuff. In 1921, Bloodgood observed that 180 patients with cancers of the tongue were tobacco users.

In 1923, Davidson and Spittle remarked that use of tobacco leaf is a common custom in Travancore, India, and described the composition of the quid as consisting of areca nut, betel leaf, winked lime and tobacco leaf, i.e. the portion of tobacco or snuff that is placed in the mouth. They commented that in addition to tobacco irritation some infection is necessary for cancer production. They further noted that buccal cancer was not observed among the Brahmin community, who lived in the same parts and who chewed fresly. They noticed that they were strictly vegetarian and such diet might have an inhibitory action on cancer.

Turner (1923) observed that the habits of betel chewing of Travancore Indians was similar to that of the people of Ceylon. The quid in Ceylon consists primarily of a medal
leaf soaked with lime, with dried tobacco and a fragment of
two or more nuts. He noticed initial signs of leukoplakia at
the site of the quid area in male patients commonly in their
fifties and sixties; female patients were affected at
ages younger than 35. He concluded that this form of cancer
was common among betel nut chewers in Ceylon.

Orr (1933) reported the geographical distribution,
dietary and chewing habits of betel nut among oral cancer
patients in Travancore. He concluded that the presence of
shell lime and tobacco in the quid as well as prolonged
retention of the quid in the mouth and a low vitamin diet
were important factors in causation of oral cancer. He made
further observations that the low frequency of oral cancer
among chewers in Fijian islands explained by the fact that the quid used in these islands contains no
tobacco.

Ahlboe (1937) reported a high frequency of pipe smoking,
tobacco chewing, snuff dipping and naswar (in Bosnia) among
oral cancer patients in Sweden. He noted that 70% of
patients with buccal, gingival and mandibular cancers have
used snuff compared to 28-37% of patients with cancers of
other oral subunits, larynx, pharynx, and oropharynx. Axel
studied squamous cell carcinomas in males, using the
Register of the Swedish Board from 1952-1971, and noted that
for two of the patients who had used snuff, the cancer
developed at the site where the snuff was placed.

In 1941, Friedell and Rementhal, reported on 8 male
patients with buccal cancer. They observed that all of them
chewed tobacco. In 1948, Khanokler noted that cancer of the
inner lining of the cheek was four times more common in 
Bosnian Hindus than in Cajamarca. He related this high 
frequency to the habit of chewing betel leaf with tobacco 
and lime and retaining it in the cheeks for several hours.

Paynter (1956) noted from analyses of 650 cases of 
oral cancer in India that 90 per cent were betel nut 
chewers. 55% of the females showed microcytic anemia, 30% 
had poor oral hygiene, 32% had leukoplakia, and 9% had syphilis.

Lundy and Harold (1961), while working in the Surgery 
Tumor Clinic of the University of Arkansas Medical Center, 
observed that patients presenting with tumors of the buccal 
and gingival areas had a long history of snuff dipping. 
They decided to study the records of patients presenting 
with intra-oral lesions for the past ten years. After 
initial examination of the data, they limited the study to 
buccal and gingival cancers and excluded cancers of the 
lips, tongue, palate and oropharynx. They found that all 
the 25 patients were males and had a long history of snuff 
use. The buccal cavity was the site of the tumor in eleven 
cases, and ten had cancers in the gingiva. The 
investigators could not exactly define the primary site for 
the rest of the cases because the tumors had involved both 
sites. All cases were exposed to snuff for more than 20 
years and most of them stated that they had always placed 
the snuff quid at the site where the tumor developed. 
History of leukoplakia was positive in most of the patients 
and showed evolution to pseudoepitheliomatous hyperplasia, 
and to early carcinoma.
Alkisson (1984) found an association between betel nut chewing (without tobacco) and cancer of the buccal cavity from analysis of 289 cases of oral cancer among the people of Territory of Papua New Guinea.

Wahi et al. (1965) studied 1,246 cases of oral and oropharyngeal cancer in Agra, India. They found that the relative frequency of oral and oropharyngeal tumors together was 31.9%. Among the oral cancer patients, the buccal mucosa was the most common site (52.0%) followed by the tongue (27.0%). They found that 90.4% of the cases either chewed tobacco alone or with pan or with lime.

Further epidemiological surveys by Wahi (1969) in the Meiningari District of India showed strong correlations between the prevalence of oral cancer and the use of local tobacco, especially when chewed.

Wahi et al. (1969) conducted a house to house survey in India and estimated the point prevalence rate of oral cancer and leukoplakia and their possible relationship to tobacco chewing. Among 56,911 adult villagers in 4 states of India, they found 26 cancer cases. The prevalence of leukoplakia ranged from 0.7% to 3.8%. A positive correlation between leukoplakia and the habit of chewing was found.

In 1970, Wahi et al. studied the prevalence rate of precancerous lesions and their possible association to chewing and other habits. In 4 villages, 3,358 persons aged 35 or above. He found 7.1% of the tobacco and precancerous lesions. The habit of chewing tobacco, especially Meiningari tobacco, was found to enhance the risk of precancerous
lesions significantly.

2 7.63 USA
Rosenfield et al (1983) reviewed 525 cases of oral carcinoma in Tennessee, USA, and compared the intraoral anatomical sites where the tobacco was habitually placed and held with other intraoral sites among tobacco chewers. He concluded that prolonged application of snuff to gingival-buccal sulcus was a factor in the development of squamous cell carcinoma at this site and that a greater proportion of females were affected.

3 2.88 Africa
A few reports investigated use of snuff among the South African Bantu. Keen et al (1985) investigated respiratory cancers and stated that snuff taking was extensively practiced and that this might account for the high incidence of carcinoma of the nose and nasal sinuses. In their opinion, the important carcinogen in snuff are 3,4-benzopyrene (strongly carcinogenic) and 1,12-benzpyrene (a weak carcinogen). Higgenson (1999) found no association between snuff and oral cancer. However, Wyke (1985), in a microscopic pathological study of oral lesions, suggested that the habit of snuff use among South African Bantu caused the lesions. The results of the histological studies suggested a correlation between snuff and oral carcinoma. In subsequent studies of oral health of South African Bantu Wyke (1971) found that leukoplakia and leukokeratosis showed highly significant correlations with tobacco habits. Homolka et al (1977) studied carcinoma of the maxillary antrum and its relationship to trace metal content of snuff. They suggested that the high concentration of nickel.
chronium, and nine might be responsible for the association between carcinoma of the maxillary antrum and smog.

In reports on oral cancer and use of Shamasah (a betel quid mixture similar to that used in Sudan) Stirling (1979) found a greater frequency of cancer of the mouth, tongue, and esophagus compared to cancer of the lower intestinal tract in Western regions of Saudi Arabia. It was stated that tobacco, alcohol, and betel nut chewing was commonly practiced in Saudi Arabia. They initially interpreted the high frequency of oral cancer as being caused by the hot climate food which is consumed in Saudi Arabia.

However, Stirling et al (1981), in a subsequent study, on cancer of the mouth in the western region of Saudi Arabia, implicated Shamasah to be the causative agent. It was concluded that Shamasah is a potential carcinogen, and the sodium bicarbonate may act as a co-carcinogen.

Salama et al (1984) examined the oral mucosa in 632 Shamasah users in various geographical regions of the Kingdom of Saudi Arabia for malignant and premalignant lesions and found that 88% had mucosal lesions compatible with leukoplakia. The lesions were found in the exact location of the quid. Squamous cell carcinoma was detected in 7 cases and carcinoma in situ in 2 cases. A causal relationship between Shamasah and development of malignant and premalignant lesion was suggested. Similar observations were made by Al Abaid et al (1988), who found that the frequency of oral cancer in the Northern regions of Saudi Arabia correlates with the use of Shamasah.
However, IARC (1985), evaluated these studies from Saudi Arabia and others. They concluded that "there is inadequate evidence that oral use of the other smokeless tobacco preparations considered (nawwa, hassaqat, mishri, al-zakhah, and shammah) is carcinogenic to humans".

A comprehensive review of smokeless-tobacco use and upper digestive/respiratory cancers was developed by IARC in 1985. The results of the studies from 1915 to 1983 were summarized in two tables. Data were provided from USA, Europe (Greece, Denmark), and Canada.

Z.Z. S.O The Sudan

Sickey (1980) was the first to report unexplained, elevated frequency of oral cancer in Sudan. El-Aswany (1982) showed that the relative frequency of oral cancer decreased as the number of biopsies increased. Other studies have also suggested an association between oral cancer and kushak use (Aboud et al. 1988); Kudid (1974) made clinical observation on the association between cancer of the lower lip and use of kushak, the kushak quid in called sufra in Arabic. The study was reported in Arabic in a form of monograph under the title 'as sufra wa saritwa a white on white'. Yech et al. (1983), Marlowe (1987) found a high relative frequency of buccal cancer in Sudan. El-Abdalla et al. 1989 reported a descriptive study on mouth and oral cancer in Sudan, they found 50 patients with oral squamous cell carcinoma, out of 82 were kushak users and cancer developed at the site where the quid was placed. It is clear that evidence for causal association available in these descriptive studies is inconclusive. Furthermore, the kushak product used in
Sudan is different from those used elsewhere elsewhere. The only exception is the product used in the Southern parts of Saudi Arabia (Sterling, 1973, 1981). Epidemiological studies by Sterling et al. 1979.
3.3 Materials and Methods

The material for this study consisted of 1885 (12.8%) neoplasms of the mouth. They were obtained from the cases registered in the Sudan Cancer Registry (SCR) during the period January 1973 to December 1985 inclusive. During this period a total of 14,922 neoplasms occurred at all body sites. Thus the neoplasms of the mouth constituted 12.6% of these cases.

During the same period a total of 10,470 malignant neoplasms occurring at all body sites were referred to the Radiation and Isotope Centre (RICK). Of these, 660 neoplasms formed (7.6%) of all mouth neoplasms. 650 were squamous cell carcinomas representing 8.2% of total body neoplasms. Eighty neoplasms were of salivary glands. Seven were odontogenic neoplasms. There were 42 Neoplasms of other histologic types, including malignant melanomas.

Cases from the two sources above (SCR & RICK) were combined and duplications were removed as shown in Table 7.

3.3.1 Material Source

The SCR was established in 1986 and its records are mainly based on data obtained from the Histopathology Department of the National Health Laboratory, and the Pathology Department, Faculty of Medicine University of Khartoum. The numbers recorded each year is approximately 1,000 cases of malignant and benign but locally aggressive neoplasms (namely basal cell carcinomas, odontogenic neoplasms and pleomorphic adenomas). The inclusion of the latter group was based on advice sought from WHO. The International Classification of Disease for Oncology.
Table 7 The distribution of neoplasms of the mouth according to histologic types during the period (1870-1985)*

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lining mucosa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinomas</td>
<td>1274</td>
<td>58.5</td>
</tr>
<tr>
<td>Malignant Melanomas</td>
<td>14</td>
<td>9.7</td>
</tr>
<tr>
<td><strong>Salivary gland neoplasms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary carcinomas</td>
<td>25</td>
<td>1.3</td>
</tr>
<tr>
<td>Adenoid cystic car</td>
<td>41</td>
<td>2.1</td>
</tr>
<tr>
<td>Mucoepid tumors</td>
<td>17</td>
<td>0.9</td>
</tr>
<tr>
<td>Adenocarcinomas</td>
<td>35</td>
<td>2.0</td>
</tr>
<tr>
<td>Acinic Cell tumors</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Benign</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleomorphic adenomas</td>
<td>144</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>Odontogenic tumors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ameloblastomas</td>
<td>143</td>
<td>7.5</td>
</tr>
<tr>
<td>Ameloblastic fibromas</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>Nymphaeas</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Adenomatoid odontogen</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>Calcifying odontogenic</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Tumors of other histologic types</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphomas</td>
<td>35</td>
<td>1.9</td>
</tr>
<tr>
<td>Fibrosarcomas</td>
<td>33</td>
<td>1.7</td>
</tr>
<tr>
<td>Other sarcomas</td>
<td>22</td>
<td>1.1</td>
</tr>
<tr>
<td>Vascular neoplasms</td>
<td>19</td>
<td>1.0</td>
</tr>
<tr>
<td>Chordrosarcomas</td>
<td>17</td>
<td>0.9</td>
</tr>
<tr>
<td>Osteosarcomas</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>Burkitt's lymphomas</td>
<td>13</td>
<td>0.7</td>
</tr>
<tr>
<td>Reticular sarcomas</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Giant cell tumors</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Metastatic tumors</td>
<td>5</td>
<td>0.3</td>
</tr>
<tr>
<td>Embryonal rhabdomyosar</td>
<td>5</td>
<td>0.3</td>
</tr>
<tr>
<td>Leukemia</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>Myxosarcomas</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Malignant histiocytoma</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
(Huk belie 1977), because most of the patients presented with the tumor in a very advanced stage.

No cases were included in which the diagnosis were based on clinical grounds alone. However, cases of leukaemias and lymphomas diagnosed from peripheral blood specimens were included. For the present study, all the cases of oral neoplasms recorded in the registry for the period of 1970-1985 were selected. One source of difficulty was that many cases were registered several times. Sometimes patients were recorded under different names or ages.

The Radiotherapy and Isotope Centre Khartoum (RICK) was established in 1967. It is the only center for treatment of patients with malignant neoplasms by radiotherapy and chemotherapy. The centre receives only histologically confirmed cases. Cases for RICK are referred from government hospitals and private clinics. Prior to admission of any patient, enquiries about habits, such as smoking, snuff taking and use of alcohol, are made. The follow up program is good and the registry is well kept. At present it is a more reliable source of information on cancer than SCR. Each patient has only one record number, which is maintained throughout the follow up period. We felt that this source is most reliable for calculation of frequency ratios. Most of the cases recorded in RICK were previously recorded in SCR.

The relative frequency of cases recorded in RICK were first described, then the total figures from both sources were combined for further analysis. Duplicates of cases recorded in both registries were removed. However, as
information on habits were available only in RICK, the
analysis of data on these characteristics were limited to
data obtained from this source. Cases of oral neoplasms
selected from RICK were for 1970-1985 as for SCR.
3.4 Results

Squamous cell carcinomas and malignant melanomas are the malignant neoplasms arising from the lining of the antrum. There were 1280 cases and they formed 97.2% of all oral neoplasms. The vast majority were squamous cell carcinomas 1274 (98.3%). Tumors of the salivary glands totalled 282 (14.7% of all oral neoplasms) 138 (49.8%) of which were malignant and 144 (51.1%) were benign but potentially aggressive (all were pleomorphic adenomas). The malignant group consisted of 53 (2.8%) adenocarcinomas, 41 (2.1%) adenoid cystic carcinomas, 25 (1.3%) squamous cell carcinomas, 17 (0.9%) adenosquamous carcinomas and 3 (0.1%) acinic cell carcinomas. Of the 192 (8.6%) odontogenic neoplasms, ameloblastomas were the most common; they formed 87.2% of all odontogenic neoplasms (5.8%). The second most common were ameloblastic fibromas. The myxomas, ameloblastic odontogenic tumors and calcifying epithelial odontogenic neoplasms formed 3.7%, 1.9% and 0.6%, respectively.

Neoplasms of other histological types, totalled 104 (9.6%). All types were represented, but lymphomas were the most common. Fibromas were the second most common.

Fig 3 Distribution of neoplasms according to age

There were differences in the age distribution of neoplasms of different histologic types. Squamous cell carcinomas were noted to increase with age. 82.1% of the cases were above the age of 40 and the highest frequency was among the age group of 50 years and above. For salivary glands, the highest frequency (30.6%) occurred in the age group of 40-50, but the frequency appeared to decline in the older age group (60 years and above).
Distribution of age by types of oral tumors

Relative frequencies

- 20-39
- 40-69
- 60
- unknown

- Glioblastoma
- Gliomatous tumors
- Other types
- Salivary tumors

Excludes melanomas
Approximately 50% of odontogenic neoplasms occurred in the young age group 20-39, while 25% occurred in the age group 40-59, and 20% in the younger age group (less than 19).

Of neoplasms of the connective and hematopoietic tissues, a greater proportion affected young individuals (83.4%, <30 years). The age distributions for the older age groups were approximately similar 14.7% and 16.4%, respectively.

Figure 4 shows distribution of various neoplasms according to sex, area of residence and tribe.

Neoplasms of different histologic categories showed differences in sex distribution. Most histologic categories were observed more commonly in males, the only exception being salivary neoplasms, while the male/female distribution was similar.

Neoplasms of different histological types showed wide geographic differences (Fig 8). Geographic and ethnic variations also existed for neoplasms of the same histological types. The results showed that all the major tribal groups were represented in this study. Squamous cell carcinomas showed the highest frequency in all tribal groups. The frequencies were relatively high among the Batiens of the north central Sudan, 20% of the squamous cell carcinomas, occurred in these tribes), Goliens (western), Nubian (northern) and Beja (eastern). The frequencies were low among the Sudano-Nuba tribes (west and
**Sex distribution of various types**

![Bar Graph](image)

- **Carcinomas**
- **Salivary tumours**
- **Odontogenic tumours**
- **Other types**
Distribution of various tumours by tribes
Distribution of various tumours by area of residence
Neoplasms of the salivary glands were the second most common. The highest frequency occurred among the Nilotic (south) and Sudanic Ruba tribes (west and south-west), compared to other central, northern and eastern tribes. The tribal distribution of the odontogenic neoplasms and neoplasms of the connective-haematopoietic tissues were similar to those of salivary glands.

The geographic distribution was very similar to the ethnic distribution. It showed a tendency to increase from south and south west towards central, northern and eastern regions. The highest rates were found in the northern and eastern regions. This trend is seen in Figure 8 which compares the relative frequencies in various geographic regions. By contrast, neoplasms of other histologic types increased from north, east and central regions towards the south and south west.

The use of khanal and smoking of cigarettes were investigated. The product of khanal used in Sudan was previously described (Ali-Hashim et al 1988). Use of khanal and cigarette by females is far less than by males. Rural females indulge more in both tobacco habits than urban females. However, women in some geographical areas (south, west, and south west) and among certain tribes (Kordofan, Hubien, Ruba), used khanal equally as men.

Of 300 cases with oral neoplasms, 292 (97.3%) used khanal. Twenty (6.4%) had used it for less than 20 years;
236 (88.6%) used it for more than 20 years and for 118 (43.0%) the duration was not recorded. Fifty-two (11.5%) smoked cigarettes, 8 (1.1%) for less than 20 years, 18 (2.0%) smoked for 20 (0.4%) more than 20 years, and for the rest the duration of smoking was not recorded.

Among the cases who used tobacco 272 (93.2%) had squamous cell carcinoma, 9 (3.1%) had salivary gland neoplasms, 11 (3.6%) had malignant neoplasms of other histological types and none of those with odontogenic neoplasms used tobacco.

For oral cancer, the significance of smokeless tobacco and smoking of cigarettes is known to be greater for squamous cell carcinoma than neoplasms of other histological types (Wynder et al 1957).

Out of 250 patients with squamous cell carcinomas and malignant melanomas of the lining mucosa, 272 (41.0%) were current users. The distribution of various neoplasms according to sex and/or histological type shows the following distribution: Squamous cell carcinoma was by far the most common among users, followed by salivary gland neoplasms.

The most common sites among users were labial, buccal mucosa and floors of the mouth. Among non-users, the palate, tongue and maxillary sinus were the most common. These differences were statistically significant. The tendency for cancers, particularly those of the lip and buccal mucosa, increased as the duration of tobacco use increased.

Smoking
Cancer at all sites studied showed a trend of increasing incidence compared to non-smokers. Among smokers of heavy cigarettes, incidence occurring at various sites increased as the duration of smoking increased.
3.5 Discussion

The main objective of this study is to provide comprehensive information on incidence of oral neoplasms in the Sudan. Inevitably, the principal measure for determining the occurrence of cancer in this country is to use frequency tables (Malik et al. 1974, 1978; Bidayat et al. 1983). The shortcomings of such epidemiological methods in general have been widely discussed (Parkin 1984), and for oral cancer in particular (Smith 1972, Pindborg 1980).

Therefore the only available measure was to estimate the relative frequency of oral neoplasms. The SCR and RICK are the only records of cancer in Sudan. Registration is believed to be complete. Probably the only cases lost are those who do not seek medical attention (Malik et al. 1974). Histological services and health care in general as well as admission to RICK were available at no cost to the patient. Therefore it is expected that the number of patients receiving histological construction and admission to RICK is reasonably high. Although it was not possible to provide incidence figures, it was felt that cases selected from these two sources would be fairly representative for the people of Sudan.

A difficulty faced while preparing this report was the absence of an accepted, uniform, topographical, behavioral, and histological classification of neoplasms of the oral cavity. The International Classification of Diseases for Oncology (WHO 1979) discussed this problem. This study included neoplasms of the maxillary sinuses because of
problems of multi-ethnic communities arising from the palate and those arising from the auxiliary anterior.

To my knowledge this study is the largest study exclusively addressing oral neoplasms from an African country. This report demonstrates the high frequency of oral cancer in the Sudan which supports earlier reports on this matter. Mawlawi (1988) while investigating 1397 malignant epithelial neoplasms in Sudan, found that 130 were neoplasms of the oral cavity: buccal cavity and lips were 51 (3.6%), tongue was 5 (0.4%) and jaw tumours (mainly ameloblastomas) were 16 (1.2%). Neoplasms of the salivary glands comprising pleomorphic adenomas were 20 (4.2%). Miryass (1997) found that the Sudan was in the lead for cancer of the larynx among the black races in Africa.

With the exception of salivary gland neoplasms, the sex distribution of oral neoplasms showed male predominance; this is similar to other reports (Pindborg 1980). The tendency of salivary gland neoplasms to occur more commonly among females has been consistently reported (Munne 1972). The age distribution for squamous cell carcinomas was similar to other reports (Pindborg 1980), showing a tendency to affect older individuals. The age distribution of the salivary glands and adenoid cystic neoplasms were similar to those reported for Africa (Munne 1982, 1984, Pindborg 1980). Similar to other reports (Muir et al 1983) neoplasms of the convective tissue affected mostly young individuals.
The present study showed that a high proportion of patients with squamous cell carcinomas of the buccal and buccal mucosa had a history of tobacco chewing. These sites are the most frequent for placement of the quid in Sudan (Edwards 1982) and are considered the most common among the population in the study area.

Evidence presented here for the association of tobacco and oral squamous cell carcinomas is supported by studies from other parts of the world (CRC 1985, WHO 1985).

Earlier studies from the Sudan suggested a possible association of oropharyngeal cancer with chewing of tobacco products (edible quid) (Ibrahim 1987). However, that among 86 patients with oral cancer, 31% had chewed tobacco, and that the carcinoma was on the site where the quid was kept. They concluded an association between tobacco chewing and development of oral cancer but causal factors were likely to be causal. The present study, however, was the earliest to report the possible association of tobacco chewing with oral cancer as the main cause. It was associated with the disease. The habit of chewing tobacco is more prevalent among men in the Sudan. In fact, the high frequency of oral cancer in the Sudan is closely linked to this habit. Tobacco chewing is considered a major risk factor for oral cancer proliferation. Our results are consistent with reports on tobacco chewing (Edwards 1982), and the use of tobacco.

This relationship is further supported by the recent findings of unusually high amounts of neoplasms identified in Sudanese patients (Mckin Son 1981).

Ethnicity and geographic distribution. People are strong indicators of cultural behavior, diet, and custom. In this study, oral neoplasms were associated with a traditional rural lifestyle of which the use of a tobacco has the highest impact. The Sudan has a wide geographical area with cultural diversity, drawn mostly from Africa and the Middle East, and fulfills the ideal environment for studying the effects of geographic and ethnic variation in the etiology of oral neoplasms.

The outstanding feature of the present report is the high frequency of squamous cell carcinoma. The tribes living in the central, northern, and western regions. On the other hand, neoplasms of other histopathological types are more common among southern, eastern, and northern tribes. The use of tobacco is widespread in the southern Sudan and northern tribes particularly Shilluk (McKin 1981).

Tribe residing in southern Sudan largely have African ethnic background but have retained their local African culture but a few have converted to Western culture (Mullik et al 1974, 1976; Reddy et al 1973). Tribes residing in northern Sudan have African ethnic background but Middle Eastern Islamic influence (Mullik et al 1974, 1976; Reddy et al 1983).
Sudan the use of loombak is not accepted among Islamic sects, Al Mahdi followers, as the habit of loombak was been prohibited by Al-Izza Al-Salafi (Abu-ulim 1999). In the south and southwest Sudan, loombak is used normally and oral use of loombak is not common.

Neoplasms of odontogenic origin, salivary glands and oesophageal tumours are more common among tribes resident in western and southern Sudan. The pattern is similar to those reported from Africa. It is believed that neoplasms of odontogenic origin are more common in Africa and in Negroid populations elsewhere in the world, (Linnell, 1986; Draisola 1979; Anand 1987). They represented 12.68% of all jaw neoplasms in Nigeria. The high frequency of Burkitt's lymphoma amongst children in many parts of the tropical world, particularly in a belt across central Africa has been established (Burkitt, 1959). This belt includes southern Sudan.

An alternative explanation is that jaw tumour including Burkitt’s lymphomas, grow slowly. It is probable that because of their slow growth and nonaggressive behaviour, they accumulate in the area while the fast lethal neoplasms were lost, due to lack of treatment and other health facilities. A high frequency of jaw neoplasms among several African nations has been reported in earlier literature, (Montgomery, 1976; Smith and Nixon 1978; Estrehan 1984; Vint 1980; Cook 1980) Sudan (Hickey 1999) South African Bantu, Higginson and Oathley 1966) Uganda (Driver 1961, 1964) Kenya (Linell1962) West Africa (Anand 1987) Nigeria (Draisola 1979).
Chapter Four
Analytical Epidemiology
1.1 Introduction

Various forms of tobacco products are used in different parts of the world. The forms of smokeless tobacco used in USA and Europe are chewing tobacco and snuff (IARC 1985, The Surgeon General, 1986, WHO 1980). The products used in Asia are tobacco with betel leaf, areca nut and lime (pan); powdered tobacco and slaked lime paste (khaini); powdered partially burnt black tobacco (mishiri); and tobacco ash and cotton or sesame oil (misri). (IARC 1985, The Surgeon General 1986, WHO 1988). Case control studies from the USA and Europe have shown that the risk of oral cancer is several times greater among snuff users than among nonusers (Morr et al 1952, Mino et al 1981). Increased risk for oral cancer has also been associated with chewed tobacco containing products in Asia (Urs et al 1973, Gupta et al 1983). However, a few reports failed to demonstrate increased risk of oral cancer associated with snuff taken orally (Higginson & Cattle 1963, Bawa et al 1977).

These reports and others have been reviewed by expert committees (IARC 1985, The Surgeon General 1986). Despite remarks made to the methodological limitations of some of these studies, both concluded that the use of snuff can cause cancer in humans, and that the use of chewing tobacco may also increase the risk for oral cancer, although evidence for the latter is not as strong.

The observation that the cancer tends to develop at the site where the tobacco mud is placed provides particularly strong evidence for a causal relationship between tobacco and oral cancer (Mino et al 1981, Hirohama 1986, Bell...
The smokeless tobacco product, used in the Sudan (toombak), is ancient and wide spread (El-bashir et al 1989). Toombak is the native name of the products of tobacco (of species Nicotiana rustica Chapl) mixed with tobacco (Nicoiw nolnestata (El-bashir et al 1969). In a study, 63% of 70 patients with oral squamous cell carcinomas from the Sudan were interviewed, and 50(61%) had used toombak (El-bashir 1969). The squamous cell carcinoma frequently developed at the site of placement of the toombak quid (El-bashir et al 1969). Studies of similar nature from Saudi Arabia suggested the etiological role of Shamma for oral cancer (Stirling et al 1981, Salem et al 1984, Al-akkad et al 1982). The present study is the first case control study to quantify the risk of developing oral cancer among habitual toombak users in Sudan.
4.2 Review of the Literature

The first case control study (Ber 1953), investigated the use of betel quid with or without tobacco among a hundred cases of cancer of the lip and hundred controls, in Trivandrum, India and found that 38 per cent of the cases practised the habit of betel quid with or without tobacco, compared to 8 per cent controls. The crude relative risk calculated by (IARC 1985) was 25.2. In a study of 100 cases with cancer of the base of the tongue, oropharynx, hypopharynx, and esophagus from Bombay, India, during the period 1962-1984, Sanghvi et al (1995) found the habit of chewing betel quid with or without tobacco, smoking and chewing of betel quid with or without tobacco; in 12 per cent, 39 per cent, of the cases respectively. Among the controls, chewing of betel quid with or without tobacco and smoking and chewing of betel quid with or without tobacco was found in 3 per cent and 24 per cent respectively. The crude relative risk for chewing of betel quid with or without tobacco, calculated by IARC 1983 (from the numbers given in the report) among 239 cases of oral cancer (comprising gum, floor of the mouth, bucal mucosa, palate and tongue), 54% of oesophagus cancer and 46% controls for all cases category was 10.2, and 4.0 respectively for both cases and controls.

Meder et al (1961) studied the medical histories of some patients with squamous cell carcinoma of the upper alimentary tract at Uppsala, in Sweden. Among the cases, 12 have cancer of the tongue; 14 lip cancer; 19 cancer of the gingiva and 8 cancer of the bucal cavity. Controls were patients having cancer of the skin, head and neck.
cancer and a few other sites. They studied the risk of multiple exposures including smoking and tobacco chewing. They found a suggestive relationship of tobacco chewing only with cancer of the buccal cavity and gingiva and not with tongue, and lip cancer. They observed that the cancer, in the majority of the cases occurred in the species of the area in which the tobacco was held. They also described the product of tobacco used in Sweden; it was a mixture of Kentucky and Virginia, usually nothing was added. They made comments on the toxicity, and stated that the possible carcinogen probably resided in the tobacco itself and did not require combustion. They made further remarks that the carcinogen found in this tobacco, in view of the few cases of oral cancer among tobacco chewers, was less potent or less concentrated than the tobacco used for smoking.

Sarna (1963) studied chewing of betel quid with or without tobacco among 239 cases of cancer of the lip, pharynx, oropharynx, and larynx and 3870 controls in Assam, India. Nearly seven per cent of the cases chewed tobacco compared to 75 per cent of the controls. The relative risk for chewing of betel quid with or without tobacco, using the figures provided by the author, was calculated by IARC (1969) as being 7.0.

Khanolkar (1963) investigated the chewing habits among 379 patients having cancer of the base of the tongue, oropharynx, and lip and a control group in Bhopal, India. Chewing of betel quid with or without tobacco, smoking and chewing of betel quid with or without tobacco and smoking (name used khatta), were investigated. The relative frequency of the habits among the cases were 12 per cent, 38
per cent and 48 per cent and among the controls 9 per cent, 24 per cent and 50 per cent respectively.

The relative risk for chewing betel quid with or without tobacco for 95 cases with oral cancer (comprising lip, floor of the mouth, buccal mucosa, palate and tongue), and 286 controls, was 8.9. In a further analysis of 276 cases of cancer of the oropharynx and base of tongue and 288 controls, the relative risk for chewing betel quid with or without tobacco was 10.9.

Shanta & Krishnamurthi (1959) studied use of betel quid without tobacco, tobacco chewing, and smoking in 208 cases of cancer of the cheeks and floor of the mouth and 278 controls in Madras, India. The relative risk for chewing betel quid without tobacco and chewing of tobacco plus betel quid without tobacco was 8.1 and 39 respectively.

In a subsequent report, Shanta & Krishnamurthi (1963) reported on the habit of chewing of betel quid without tobacco, and chewing of tobacco and betel quid without tobacco, among 262 cases of cancer of the lip oropharynx, nasopharynx and tongue, and 400 controls. The crude relative risk for chewing betel quid without tobacco for males and females were 8.0 and 8.0 respectively, and chewing of tobacco and betel quid for males and females were 17.2 and 20.1 respectively.

Wahi et al. (1965) in a very informative study, investigated tobacco chewing and smoking in one thousand nine hundred and sixteen cases of oral and oropharyngeal cancer. They provided a detailed description of the habit
used the Indians. Tobacco was either chewed alone or with 
pan (betel leaf, betel nut, and lime or with lime alone).
The grid was placed in the buccal gingival fold of either 
side, for variable periods of time and occasionally at night 
also, or else tobacco was smoked and one of the following 
was used: bidi, chilam, hookah, or cigarette. Occasionally 
tobacco is used in combination. Tobacco was also used for 
chewing and smoking by the same individual.

The study group included high a proportion of Khari 
over 90% cases with cancer of the lip, tongue, and 
tonsils, and 1010 controls in ages, India, during the period 

The analysis of tobacco use by sex and site showed that a 
large proportion of women who were both chewers and smokers 
suffered from carcinomas of various oral sites. The 
percentage of women showed gradual increase among women of 
lip and buccal, anterior two-thirds of the tongue, gingiva, 
palate, tongue posterior third and tonsillar carcinoma. A 
fair number of cases of buccal and gingival carcinomas were 
observed. Statistical analysis showed a significant 
association of all tobacco habits with oral and 
pharyngeal cancers at all sites with p square value 
< 0.05 (< 0.05). Chewing alone was found to be significantly 
increased in cancer of buccal and anterior two thirds of 
the tongue with p square value 8.4 (p < 0.05), 5.2 
(p < 0.05) respectively. The smoking habit was significantly 
associated with cancer of anterior two thirds of the tongue, 
and buccal mucosa with p square value of 3.2 (p < 0.05) 
and 6.4 (p < 0.05).
Among females, all those having lip cancer were tobacco chewers. Chewing habits among females correlated with the number of smokers at each site. A very few smokers had cancer at sites other than palate and posterior third of the tongue. Analysis of all habits of tobacco showed a significant relation with oropharyngeal cancers with a quasi-square value of 253.6 (P<0.05). When the incidence of oral and pharyngeal cancer was analyzed by site in each group of different tobacco users, chewers, smokers or both, the frequency of cancer of lip remained similar among different tobacco users, smoker of the buccal cavity was the most frequent among all users.

In males buccal cancer was the most common among patients who were both smokers and chewers and then smokers only. Carcinoma of the anterior two thirds of the tongue showed a reversed trend, smokers being the highest, followed by combined habits and lastly the chewers. Similar trends in cancer of the tongue, but entirely.

Female chewers suffered from cancer of the buccal mucosa than cancer of the anterior two thirds of the tongue and gingiva. Majority of patients who did use tobacco had cancer of the anterior two thirds of the tongue.

Analysis of 136 of oral leukoplakia by site showed that the highest incidence for patients in the buccal mucosa membrane while its frequency in the tongue was low. Seventy three per cent of the cases chewed tobacco, 33 per cent chewed tobacco and smoked, and 55 per cent were smokers. 3.8 per cent were alcohol users over a varying number of years. All cases were addicted to tobacco and had
poor oral hygiene. They summarized their findings; the buccal mucosa was the most common site (52.3) of oral and oropharyngeal cancer in southern India. The peak incidence in both sexes was between 50-54 at all sites, and cancer of the buccal cavity occurred at earlier age compared to other sites. A male to female ratio of 2.3:1 was found and this was related to differences in tobacco chewing. A large proportion of the cases were tobacco chewers. Low serum vitamin A was found in 70.2 per cent of cases and it was considered as an adjuvant in the carcinogenic process. SYPHILIS and alcohol showed no association with oral and oropharyngeal cancer. Poor oral hygiene was thought to be a contributing factor.

Mitra and Mitra (1938), conducted a very large epidemiological study of oral and oropharyngeal carcinomas in central and South East Asia. They provided information on mortality due to buccal and pharyngeal cancers (using a comparison of age and sex standardized mortality rates, per 1,00,000 of patients suffering from cancer of the buccal and pharynx in selected countries 1939-1959, using sex and age composition in Bombay and Ceylon).

The highest rates for cancer of the buccal cavity and pharynx were found in Bombay and Ceylon, compared to low rates in Europe and Japan the only exception being France, Ireland, and Switzerland, where a moderate increase was recorded. The relative frequencies of oral cancers as percentages of total body cancers for various places in India and Ceylon were given. The figures for Bombay (Southern India), Manipur (Northern India), Jaffna (Ceylon), were 77.92, 87.92, 88.12 respectively. The site
distribution of oral cancer also showed regional differences. The predominant site in all places was the cancer of the buccal cavity; the relative frequency of this cancer out of total sites was as high in Colombo, Vellore, and Bangkok. Cancers of the floor of the mouth and anterior two-thirds of the tongue were noted to be much more frequent in AitkenAitken, among the AitkenAitken. Cancer of the lip was exceptionally high in Bhutan and Bangkok.

Be provided the relative frequencies of the intra-oral sites among tobacco chewers. Cancers of the buccal mucosa, lip, and anterior two-thirds of the tongue were the highest respectively. The risk was not increased for cancers of the posterior tongue and oropharynx among tobacco chewers. Sites showing increased frequencies for smokers were oropharynx, posterior tongue and anterior tongue. We concluded that, such increase among Central and Southeast Asians, indicated the existence of strong environmental factors in these areas and suggested the influence of commonly practiced habits.

Another report by Hirayama (1988) investigated patients with oral and oropharyngeal cancer and a control group of patients with other diseases. The report was adequately evaluated by IARC (1988) and the relative risk for the various combinations were calculated (from the authors given in the report).

Steve N Krugda (1970) studied chewing of betel quid with or without tobacco in 111 cases of cancer of the oropharynx,
and 1000 controls in Sri Lanka. The crude relative risk for chewing betel quid with or without tobacco was 9.9.

Jagjwala and Boopahade (1971) evaluated the cancer risk among betel quid chewers and smokers in Bombay, India. The study group comprised cases of cancer of the oral cavity, pharynx, larynx and esophagus and an equal number of controls, which were matched for age, sex and religion. The relative risk for cancer at each site in chewers and nonchewers was estimated. The relative risk is found to be high for all cancer sites studied, but higher for cancer of the oral cavity. The relative risk estimated for chewers only, chewers and smokers, and smokers only, was found to be significantly high for all cancer sites studied, the only exception being cancer of the larynx. A relative risk of 7.7 was found to be associated with cancer of the buccal cavity, among chewers compared to non-chewers; the cancer predominantly arose in those who placed the quid in the gingivolabial groove, from a few minutes at a time to overnight. In the case of the hard palate, the alveolus, and the anterior two-thirds of the tongue, the contact was maintained for shorter periods of time.

In a subsequent report Jagjwala (1977) presented further analysis of the data reported by Jagjwala and Boopahade (1971) relating to the separate as well the combined habit of chewing and smoking, and also examined the data for possible synergism. The study group consisted of 250 cases of patients with oral, pharyngeal and esophageal cancer and an equal number of controls. He concluded that 70 per cent of cancers of esophagus and 75 per cent of cancer of the hypopharynx were attributed to synergistic effects of
Martinez (1989) in a community-based case-control study of 400 cases with cancer of the oral cavity, pharynx and esophagus in Porto Rico, each case was matched with three controls for age and sex. One from the hospital where the case was diagnosed and the remaining two from the neighborhood where the case lived. 1 200 age and sex
matched controls were interviewed. Dietary factors such as fresh fruits, vegetables, green egg and milk were deficient in both cases and controls. There were significant differences among cases as compared to controls regarding use of alcohol, tobacco, hot beverages (mainly coffee) and spices. The habit of chewing tobacco mixed with saltwater were not significantly different among cases and controls. The smoking group of IARC (1985) calculated the relative risk for those cases with only a chewing habit as compared to those without habit. The relative risk for men was 11.5 for oral cancer, 6.7 for cancer of the pharynx and 1.2 for esophageal cancers.

Khanna et al (1975) reported on chewing of betel quid with or without tobacco in 208 cases of cancer (sites not specified) and 100 controls in Varanasi, India during the period (1965-1970). The crude relative risk was 27.9. In another study from India, Nalaw & Sanghvi (1976) reported a relative risk of 4.2 among chewers of betel quid with or without tobacco (calculated by IARC 1985 from the numbers given in the report) from study of 214 cases and 238 controls in Bombay.
The study from Minnesota, by Nance et al. (1952, 1953) investigated the habit of tobacco use among a group of 40 white males with intra-oral carcinomas, 72 having carcinomas of the lip, 23 carcinomas of the face and 23 oral leukoplakias and compared these to a control group consisting of 38 patients with benign and other benign diseases. The relative risk associated with the exposure of more than 20 years of tobacco use as compared to less than 20 years was 2.4 for carcinomas of the face, 2.8 for lip cancer, 4.0 for intra-oral carcinomas and 7.8 for leukoplakias. For the same study, The Surgeon General (1964) calculated a peak relative risk for mouth cancer using smokeless tobacco about 4.9 (1.0, 10). They provided a partial explanation for the apparent lack of effect of other forms of tobacco use by the possible confounding effects between smokeless and smoked tobacco. Wynder et al. (1957) examined a variety of risk factors including the use of chewing tobacco among those with squamous cell carcinomas of the upper gastrointestinal tract. They found an increased risk associated with the duration of chewing tobacco for carcinoma of the gingiva and oral cavity, but not for cancer of the tongue or lip. From further studies, Wynder et al. (1957) found that more patients with cancer of the oral cavity than controls chewed tobacco.

The data that have been published with regard to smokeless tobacco use as a possible carcinogenic factor for intra-oral carcinomas, usually referred to the entire oral cavity and often the pharynx. However, a few reports, have investigated the gingival/buccal areas, the site area since the snuff is placed separately from contiguous areas such as tongue, lip, and floor of the mouth.
Boschkefeld (1961) using clinical observations, drew attention to the fact that cancer occurs at the site of application of the snuff and to the importance of limiting the study of the role of snuff to the etiology of carcinoma of the oral cavity, to specific intramural sites, where the tobacco snuff is placed rather than taking the entire oral cavity into consideration. He studied squamous cell carcinomas arising at four intramural sites, anterior two-thirds of the tongue, floor of the mouth, the gingiva and buccal mucosa. To allow comparison of gingival and oral carcinomas which arise at or near the site of application of the snuff with those which arise at the tongue and floor of the mouth adjacent to but in direct contact with the snuff. He divided the four sites arbitrarily into two groups gingival/oral cancers as cases and the tongue and floor of the mouth as controls. The cases comprised 301, the controls 225. He found that 30 per cent of the female cases were habitual snuff users as compared to 21 per cent of the controls. The conclusion that, the use of snuff, by placing it in the lower gingival groove, was a habit employed by an appreciable number of people notably women in the U.S. A.,a greater proportion of women presented with carcinoma at this site compared to other areas of the mouth, where the use of snuff not less frequent. The data suggested that prolonged application of snuff into the gingival groove was a factor in the development of cancer at this site.

Peacock et al. (1968) compared the history of tobacco use among 50 cases of cancer of specific intramural sites baseal, alveolar ridge and floor of mouth with two controls.
groups consisting of 168 in patients with diagnoses other than cancer, and 317 outpatients. The relative risk was estimated to be 2.0. The study was reviewed by a working group of the Surgeon General and remarks were made that the relative risk was probably underestimated and that the limitation of the data did not permit evaluation of the risk of mouth cancer related to the duration of use of smokeless tobacco. Vaiger et al. (1971) assessed the risk of cancer of the mouth using 333 cases of mouth cancer (lip, buccal, tongue, palate, pharynx, sinonasal, salivary glands, pharynx, and larynx, with three control groups consisting of 218 patients having mouth diseases other than cancer, 584 patients with cancers of sites other than mouth, pharynx or larynx, and 747 patients having no cancer. They examined the occupation, history of syphilis, nutritional deficiencies, family history, presence of sharp or broken teeth, fitness, and duration of wearing dentures; use, type, amount and duration of use of tobacco; use, amount and duration of use of alcohol. 31.4 percent of the urban males of the cases have used tobacco in one form or other up to 4 years. This was significantly greater than group 3 and 4 but not different from group 1. Among the users of tobacco, the proportion of 25 or more years of tobacco use was higher in group 3 than in group 2, 3 and 4. There was a greater proportion of cigarette smokers in group than in the other study groups. Great proportion in group 1 used alcohol compared to other groups, but the difference was not significant.

Zaharias and Horak (1977) using personal interviews obtained from 7,518 incident cases of invasive cancer from the population based Third National Cancer Survey, recorded
the quantitative lifetime use of cigarettes, cigar, pipe, 
unsewmed tobacco, wine, beer, hard liquor, and combined 
alcohol as well as education and family income.

They compared one cancer site with controls from other 
sites to test the association of site of cancer with the 
exposure. The results of analysis of intracranial tumors were 
provided for cancer of the lip tongue as one group compared 
with cancer gum and buccal cavity. A significant positive 
association with tobacco classified as (cigarette pipe, and 
chewing snuff) were found for gum and buccal cancers. 
Cigarette smoking were associated positively with lip-tongue 
cancers. They concluded with the following statement ' For 
cancer of the gum and buccal showing of tobacco and snuff 
use to play the strongest association' they explain this 
statement by the fact that both forms of tobacco are often 
held in prolonged contact with the gum and buccal mucosa 
during use; this allows ample opportunity for exposure to 
carcinogens

Nylander (1977) compared 3,718 patients with cancer of the 
lung, stomach, esophagus and bladder with over 12,000 
controls. He found increased risk among smokers. The risk 
of small tumor, and esophageal cancer were increased with 
the quantity of alcohol consumed. Williams et al (1983) 
studied the use of tobacco and other factors in 7,000 
incidence cases of cancer from a population based third 
National Survey in United States. They compared each 
specific cancer site with control from other sites to test 
the association with the exposure variables. They found a 
significant association with cigarette smoking and cancer of 
head, larynx, oral cavity, esophagus, stomach, bladder,

Other forms of tobacco use were associated with cancer of the oral cavity, larynx, larynx, lung, and cervix. Using a case-control study in North Carolina to assess the relative risk of oral and oropharyngeal cancer due to small dipping among 255 cases and 552 controls, Viano et al. (1981) found a relative risk of 1.2 (2.5, 0.7) among white, non-smoking females. The risk approached a 50-fold increase for smokers of the gums and buccal mucosa, among chronic users.

Brown et al. (1977) studied 75 cases of oral cancer recorded during the period 1957-1971, with control groups matched for sex, age, primary occupation, and residence identified from a clinical practice in the UK. They found a negative association between users and controls regarding the use of chewing tobacco. The IARC (1985) reviewed the report and noted that the negative association may be due to confounding by secondary occupation. There was matching on primary occupation in the study design, so the negative association disappeared when data was stratified by occupation. The IARC made further observations about differences in time of ascertainment of the cases (1957-1971) and of the controls (1974-1976) resulting in matching of obtained age at different time intervals, bias due to interviewing of next of kin of deceased cases and oversampling by neighborhood and occupation.

Recently Gupta et al. (1990) assessed the preneoplastic potential of oral precancerous lesions in the Betelnic. In India, in a cohort of 12,212 tobacco users, 190 (0.16%) individuals developed oral cancer over a period of 8 years, 66 (1.0%) of these arose from preexisting precancerous
lesions. Of the premalignant lesions, nodular leukoplakia showed the highest malignant transformation rate. Malignancy developed in six of 13 having nodular leukoplakia followed over period of 2.3 years (mean). The highest relative risk of 3243.2 was found among individuals with nodular leukoplakia even when compared with individuals with tobacco habits but without premalignant lesions. Relative risk of 397.3, 303, 43.3, 23.3, 12.8, were found among other premalignant lesions, submucous fibrosis, others [non-specific diagnoses such as red areas, ulcers, and benign growth], leukoplakia, homogenous leukoplakia, and lichen planus respectively.

Sankararayanan et al (1989) carried out a case-control study of the risk due to use of pan (betel)-tobacco chewing, bidi and cigarette smoking, drinking alcohol, and using snuff, among males with cancer of the gingiva in Kerala, South of India. Only pan-tobacco chewing was investigated in females. They studied 187 cases and 690 hospital based controls. A significant positive association (P<0.001), except for snuff use (P=0.05) was found for all exposures studied including the pan-tobacco chewing in females. A risk of 15.07 was associated with chewing of ten or more quids per day, and the frequency of chewing per day was the strongest predictor of risk in males. A relative risk of 18.09 was found among females. In males a relative risk of 3.38, 2.02 and 3.9 was found among smokers of more than 20 cigarettes per day, regular use of alcohol, and snuff respectively.
4.3 Materials and Methods

4.3.1 Case Control Study

The Radiotherapy and Isotope Center Khartoum (RICR) is the only hospital in the Sudan for treatment of patients with malignant neoplasms. A questionnaire is routinely administered to all hospital admissions (Appendix I). The questionnaire includes information on habits, such as use of kusha, smoking of cigarettes, and alcohol consumption.

Two groups of cases were studied. One consisted of 200 cases with squamous cell carcinomas of the lip, floor of mouth, and buccal mucosa (International Classification of Diseases for Oncology 1976), rubrics: 141.3, 143.9, 144.9. These patients were retrieved from the files of the RICR during the period 1970-1985. These neoplasms arose from sites of preference for kusha abuse, buccal mucosa and floor of mouth for placement of kusha yield among Sudanese (Dorothy et al. 1988).

Patients admitted to the same institution during the same period of time, 271 cases with squamous cell carcinomas of the tongue, palate and maxillary sinuses (International Classification of Diseases for Oncology 1976) rubrics 141.5, 145.5) constituted the second group of cases. The patients in these patients arose from oral sites with little or no direct contact with the kusha yield.

Individuals admitted during the study period for treatment of non-squamous oral neoplasms, and for neoplasms of non oral sites unrelated to tobacco use, were retrieved from the files of RICR constituted one control group. They included 23 epidermoid tumors, 50 salivary gland neoplasms, 2 malignant melanomas, 81 non-epithelial tumors, and 18
neoplasms of the breast and head and neck. Volunteers attending oral health education programs conducted in various regions of the Sudan were selected as a control group to provide an estimate of the use of toothbrush among disease-free individuals. A questionnaire similar to the one used in (R.C.R) was used by the interviewers (Appendix 2).
Case control 2

Despite the fact that the above study used two case groups and two control groups and obtained consistent results of increased risk across both study groups, information in hospital records has inherent limitations, as records are often incomplete and do not consistently provide the desired information. Furthermore, the questionnaire which is routinely administered for all hospital admissions did not include detailed information about the use of khat, smoking and alcohol. Therefore the present study was undertaken to examine the risk associated with use of khat using data obtained by more detailed interviewing of cases and controls.

Identification of cases

A questionnaire was designed (Appendix 2) and all new histologically confirmed cases of squamous cell carcinomas of the mouth, ICD-O (160, 143 145) World Health Organisation 1988, presenting to Khartoum Teaching Dental Hospital, during the period 1985–1989, were interviewed for history of khat, frequency per/day, quantity used per/week, type of the product used, and the place of application. Questions were also asked about smoking of cigarettes and alcohol use, information about age, sex, tribe, residence, and clinical description of the lesion were also obtained.

Identification of controls

Prospective controls were identified from relatives of the patients' close associates of the patient and matched for sex, age (within 5 years), and tribe. If a suitable control was not found among the associates of the patient, a control
was sought from the hospital admission. A total of 10 controls were ascertained.

Statistical Analysis:

For both studies the SPSS/C+ (a computer software) was used to describe the frequency distribution of selected characteristics of the study population such as age, sex, and other attributes to assess the comparability of the study groups, using cross tabulations, scatterplots, and histograms. The object of each preliminary descriptive analysis was to examine correlations between variables and the identification of any outliers, extreme values, or clusters important in subsequent analyses.

A crude exposure-disease association was initially performed by calculation of odds ratios for single 2x2 tables for each of the exposures of interest. Stratified analysis with adjustment for confounding variables was carried out to further examine hypothesized causal pathways between hongshak and squamous cell carcinoma. Evidence of a relationship between duration of use of hongshak had also been examined. Because many variables in addition to the exposure of interest may show a positive or negative association with squamous cell carcinoma (i.e. tribe), all analyses were done separately for each variable, in order to determine whether relationships are likely to be a) causal (b) not causal, but due to an association with underlying causal variables, or c) an artifact of improper study design or analysis.

After a thorough initial investigation using stratified analysis, a more complex analysis using multivariable
Techniques have been used to assess the independent contribution of each exposure of interest. Although stratification allows for a control of a few confounding factors, control for multiple confounding factors becomes inefficient. With respect to hookah consumption behavior, it has been shown that persons who indulge in the habit also smoke, therefore a mathematical model using variables suggested by previous stratified analysis has been constructed, using Efrat (a computer software which was developed by the Department of Biostatistics of University of Washington, Seattle, USA). Unconditional logistic regression was used to assess the risk (probability) of oral cancer development during the specified time interval as a function of the independent variables...

The exposure of association between hookah use, smoking of cigarettes and alcohol use and oral cancer was the relative risk (RR), approximated by the odds ratio (Cochrane, 1952). Estimates of the RR and confidence intervals were calculated for each of the variables stratified using 2x2 tables and summary of the results was obtained using the Mantel-Haenszel method (1959). Unconditional logistic regression was used to estimate the relative risk and adjust simultaneously for multiple variables and to test for interaction between risk factors. Population attributable risk for use of hookah un

...
4.5 Results

4.5.1 Case-control I

Comparison of cases and controls by various demographic characteristics, age, sex, place of residence, and tribe is presented in Table 15. Control group 1 tended to be younger than the cases. The majority of the cases and controls were in the age (2 group 30-69), except control 2 the majority were elderly in the age (group 70+).

The cases and controls were of similar sex distribution. There were more males, the range of ratios being 1.4 (females):1.8 (males), the only exception being case group 2 which contained approximately equal number of males and females.

The distribution of place of residence among cases and controls show that all the geographical zones were fairly represented, the only exception being that there were no individuals for Southern Region in control group 2.

The tribal distribution is very similar to place of residence. The goaali, maloon, bila, and jokhoo, are northern, eastern, central tribes respectively and are ethnically similar. While the northern, nilitro, nuna are eastern, southern and north western tribes respectively.

Toothuk was used by 218 (79.5%) cases and. 21 (11%), 59% (21%) controls respectively. 19.7% and 19.7% of the cases were smokers, compared to 19.3% and 17.5% controls respectively, and was slightly more common among case group 1 and control group 2. 95% and
<table>
<thead>
<tr>
<th>Category</th>
<th>Control1</th>
<th>Control2</th>
<th>Control3</th>
<th>Control4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le 0-10</td>
<td>17</td>
<td>9.3</td>
<td>513</td>
<td>18.2</td>
</tr>
<tr>
<td>10-20</td>
<td>21</td>
<td>19.3</td>
<td>623</td>
<td>21.1</td>
</tr>
<tr>
<td>20-30</td>
<td>33</td>
<td>16.2</td>
<td>603</td>
<td>21.4</td>
</tr>
<tr>
<td>30-40</td>
<td>39</td>
<td>19.1</td>
<td>418</td>
<td>14.6</td>
</tr>
<tr>
<td>40-50</td>
<td>64</td>
<td>14.4</td>
<td>370</td>
<td>13.4</td>
</tr>
<tr>
<td>50-60</td>
<td>10</td>
<td>8.3</td>
<td>65</td>
<td>20.2</td>
</tr>
<tr>
<td>60+</td>
<td>11</td>
<td>5.4</td>
<td>21</td>
<td>2.4</td>
</tr>
<tr>
<td>SEX</td>
<td>103</td>
<td>50.5</td>
<td>978</td>
<td>34.7</td>
</tr>
<tr>
<td>N</td>
<td>236</td>
<td>49.5</td>
<td>161</td>
<td>55.3</td>
</tr>
<tr>
<td>Residence</td>
<td>124</td>
<td>51.3</td>
<td>1097</td>
<td>38.9</td>
</tr>
<tr>
<td>Eastern</td>
<td>48</td>
<td>12.8</td>
<td>55</td>
<td>12.3</td>
</tr>
<tr>
<td>Central</td>
<td>95</td>
<td>25.6</td>
<td>58</td>
<td>21.0</td>
</tr>
<tr>
<td>Kordofa</td>
<td>31</td>
<td>8.3</td>
<td>53</td>
<td>26.0</td>
</tr>
<tr>
<td>Darfur</td>
<td>33</td>
<td>9.9</td>
<td>54</td>
<td>26.2</td>
</tr>
<tr>
<td>Southern</td>
<td>33</td>
<td>9.9</td>
<td>54</td>
<td>26.2</td>
</tr>
<tr>
<td>Darfur</td>
<td>26</td>
<td>12.7</td>
<td>42</td>
<td>1.5</td>
</tr>
<tr>
<td>Sudan</td>
<td>11</td>
<td>4.0</td>
<td>36</td>
<td>1.2</td>
</tr>
<tr>
<td>Total</td>
<td>320</td>
<td>67.7</td>
<td>212</td>
<td>38.3</td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>12.3</td>
<td>29</td>
<td>19.7</td>
</tr>
<tr>
<td>Total</td>
<td>376</td>
<td>94.1</td>
<td>241</td>
<td>33.6</td>
</tr>
</tbody>
</table>

*Case1*: Lip, Floor of mouth and buccal cavity
**Case2**: Maxillary sinus, Tonsils, and Palate
***Control1**: Tumours other than carcinomas
****control2**: Healthy individuals from the general populations
The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The relative risk of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

Table 2 about estimates of the relative risk intervals for one of the cases which a portion of the logistic model. The rate was estimated by controlling for the binomial relative risk for the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The relative risk of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

Table 2 about estimates of the relative risk intervals for one of the cases which a portion of the logistic model. The rate was estimated by controlling for the binomial relative risk for the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

Table 2 about estimates of the relative risk intervals for one of the cases which a portion of the logistic model. The rate was estimated by controlling for the binomial relative risk for the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

Table 2 about estimates of the relative risk intervals for one of the cases which a portion of the logistic model. The rate was estimated by controlling for the binomial relative risk for the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

Table 2 about estimates of the relative risk intervals for one of the cases which a portion of the logistic model. The rate was estimated by controlling for the binomial relative risk for the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

Table 2 about estimates of the relative risk intervals for one of the cases which a portion of the logistic model. The rate was estimated by controlling for the binomial relative risk for the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

Table 2 about estimates of the relative risk intervals for one of the cases which a portion of the logistic model. The rate was estimated by controlling for the binomial relative risk for the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

The relative and tribe were the RR for men at the ages, and 1.00 was not found in the first case group. The rate of use in both was seen out of the rate of redoubt for more than 10 and significantly increased in the

loombak users among the control groups were 11.3% and 26.8%. Based on the corresponding relative risk estimates of the case groups 7.3, 3.8, the estimates yielded population attributable risk of 41.6%, 43.8%, respectively Cole & Naesschan (1971). Thus more than one third of occurrence of oral cancer in the Sudan can be attributed to the use of loombak.

4.5.2 Cases control 2

Interviews with 114 cases and 93 controls was completed. The general characteristics of cases and controls, is shown in Table 10.

Controls tended to be younger than the cases, and they were more females. The case control distribution of residence were similar for North and Center of the Sudan. There were more cases from the East and South and fewer cases from the West compared to controls. However, the distribution of the cases and controls by tribe were fairly similar. Smoking was more frequent among cases than among the controls. All smokers were cigarette smokers. Among cases 49 (43%) were smokers compared to 32 (34%) of controls. Among smokers 13 (26.5%) smoked for more than 10 years compared to 6 (16.6%) among controls. Alcohol use compared by 40 cases (42.1%) and 20 controls (21.3%).

Table (11) shows estimated crude, stratified and adjusted relative risk associated with loombak use, smoking, and alcohol consumption. Among the cases, 82 (71%) used loombak compared to 28 (30.6%) among the controls. Of the cases, 54 (65.9%) used loombak for more than 10 years compared to 6 (20.7%) of the controls. Among users, 33 (40.2%) of the
Table 2: Relative risk for duration of exposure to tosmak*

<table>
<thead>
<tr>
<th>Duration</th>
<th>No of Subjects</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>case1</td>
</tr>
<tr>
<td>no 198</td>
<td>213</td>
<td>1.0</td>
</tr>
<tr>
<td>ever 215</td>
<td>92</td>
<td>5.2</td>
</tr>
<tr>
<td>&lt;20ys 19</td>
<td>10</td>
<td>6.1</td>
</tr>
<tr>
<td>&gt;20ys 120</td>
<td>23</td>
<td>20.6</td>
</tr>
<tr>
<td>Unknown 90</td>
<td>56</td>
<td>4.0</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, and tribe
**Relative risk and confidence intervals were estimated for the two control groups.
cases slept with quid in their mouth. Similarly, among the controls 4 (13.8%) have slept with quid in their mouths. Of the cases 37 (45.1%) habitually retained quid in their mouths for more than 10 minutes compared to 3 (10.3%) of the controls. The frequency of use of tolkombak per day was also more common among cases than controls. Among the cases, 38 (46.3%) used tolkombak more than 10 times a day compared to 11 (37.9%) of the controls.
### Table 10 Case Control Characteristics

<table>
<thead>
<tr>
<th>Age</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;29</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>30-39</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>40-49</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>50-59</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>60-69</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>70+</td>
<td>25</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>47</td>
<td>27</td>
</tr>
<tr>
<td>Male</td>
<td>87</td>
<td>86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residence</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td>Eastern</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Center</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>West</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>South</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilotic</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Northern</td>
<td>37</td>
<td>52</td>
</tr>
<tr>
<td>Sohima</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>Sudanic</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker</td>
<td>64</td>
<td>81</td>
</tr>
<tr>
<td>Smoker</td>
<td>40</td>
<td>32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of Smoking</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>45</td>
<td>51</td>
</tr>
<tr>
<td>&lt; 10 times</td>
<td>36</td>
<td>26</td>
</tr>
<tr>
<td>&gt; 10 times</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non user</td>
<td>66</td>
<td>73</td>
</tr>
<tr>
<td>user</td>
<td>49</td>
<td>20</td>
</tr>
<tr>
<td>Variable</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>Facebook</td>
<td>32</td>
<td>66</td>
</tr>
<tr>
<td>Male</td>
<td>52</td>
<td>28</td>
</tr>
<tr>
<td>Duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 years</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>≥10 years</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>In the mouth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 minutes</td>
<td>59</td>
<td>26</td>
</tr>
<tr>
<td>≥10 minutes</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>Sleeping with cover</td>
<td>48</td>
<td>25</td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>Frequency per day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 times</td>
<td>44</td>
<td>16</td>
</tr>
<tr>
<td>≥10 times</td>
<td>38</td>
<td>11</td>
</tr>
</tbody>
</table>

*All adjusted for age, sex, tribe, residence, smoking and alcohol consumption.*
There was excess risk for squamous cell carcinomas among toszobak users RR 5.7 (95 per cent confidence intervals 3.0-10.8). Possible confounding of the results by demographic characteristics was evaluated. The relative risk showed trend of an increasing trend with age. There was a very low risk in the younger age group <30. The relative risk increased in the age group 40-49 but was not statistically significant RR 3.6 (95 per cent confidence intervals 0.6-20.4). The RR was statistically highly significant and showed trend of increase with age among users of age 50 and older. P value was <0.001. The RR reached twenty-eight-fold at age 70 and older, RR 28.0 (95 per cent confidence intervals 2.3-772.6). Estimates of RR adjusted for age by Mantel-Haenszel method (1959) (M-H) was 5.6 (95 per cent confidence intervals 2.9-10.6).

There was twice excess risk in females compared to males. The RR adjusted (M-H) for sex was 6.6 (3.3-13.0). There were excess risk in all geographical areas studied. However, in the South due to empty cells it was not possible to calculate the RR and confidence intervals. The highest RR was found in the western and central regions, while the lowest was in the eastern region. There was risk associated with toszobak use in all tribal groups. It was not possible to calculate RR associated with toszobak use among northern tribes, due to empty cells. The highest RR was in the northern tribes and the lowest was in the western tribes. The RR adjusted (M-H) for tribes was 5.8 (95 per cent confidence intervals 3.0-11.5).

The RR for use of toszobak was higher among non-smokers than among smokers. In non-smokers 6.3 (95 per cent
confidence intervals 2.7-15.2) and 5.1 (1.7-16.4) among smokers. Alcohol did not alter the RR among users and non-users of toombak.

The RR associated with toombak use, controlling simultaneously for age, sex, residence, smoking and alcohol use is shown in Table (12). Controlling simultaneously by logistic model for all the above variables did not alter significantly the RR 5.5 (2.6-11.7) and was very close to the crude. However, the RR was significantly increased among users of toombak for more than 10 years, 10.7 (95 per cent confidence intervals 6.4-60.7), use of toombak for than 10 times per day 8.3 (3.1-22.8), and habitual retainers of toombak in the mouth for more than 15 minutes 27.2 (95 per cent confidence intervals 8.4-114.7). The RR associated with sleeping was lower than the crude and adjusted RR 4.2 (95 per cent confidence intervals 1.3-13.6).

Evaluation of possible interaction of risk factors is shown in Table (13). Low relative risk was estimated among individuals who smoke and use toombak and individuals who drink alcohol and use toombak, RR 0.9 (95 per cent confidence intervals 0.15-5.2) and 0.8 (95 per cent confidence intervals 0.45-15.2) were estimated respectively. An elevated RR was found among non-smoking and alcohol users but toombak user 5.8 (95 per cent confidence intervals 2.3-15.3). Among non-smokers, non-toombak users but alcohol consumers the RR was 4.8 (95 per cent confidence intervals 1.0-22.8). Among smokers who didn't use alcohol or toombak the RR was unity, 1 (95 per cent confidence intervals 0.24-4.4).
Table 12 Case Control Characteristics, Relative Risk and Confidence Intervals

<table>
<thead>
<tr>
<th></th>
<th>Cases No</th>
<th>Zs</th>
<th>Controls No</th>
<th>Zc</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco</td>
<td>Non-users</td>
<td>32</td>
<td>65</td>
<td>32</td>
<td>1.9</td>
<td>(0.9-13.9)</td>
</tr>
<tr>
<td></td>
<td>Smokers</td>
<td>82</td>
<td>29</td>
<td>82</td>
<td>5.7</td>
<td>(0.0-0.13.9)</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;20</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>1.5</td>
<td>(0.1-13.8)</td>
</tr>
<tr>
<td></td>
<td>20-29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.8</td>
<td>(0.1-14.5)</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>11</td>
<td>17</td>
<td>11</td>
<td>3.0</td>
<td>(0.6-23.4)</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>37</td>
<td>27</td>
<td>37</td>
<td>3.7</td>
<td>(1.4-13.9)</td>
</tr>
<tr>
<td></td>
<td>50-69</td>
<td>26</td>
<td>23</td>
<td>26</td>
<td>12.6</td>
<td>(2.7-55.3)</td>
</tr>
<tr>
<td></td>
<td>70+</td>
<td>25</td>
<td>8</td>
<td>25</td>
<td>28.6</td>
<td>(2.3-772.8)</td>
</tr>
<tr>
<td>M R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3</td>
<td>(2.9-46.9)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>47</td>
<td>27</td>
<td>47</td>
<td>11.1</td>
<td>(2.9-46.9)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>67</td>
<td>68</td>
<td>67</td>
<td>5.2</td>
<td>(2.3-11.0)</td>
</tr>
<tr>
<td>M H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.6</td>
<td>(3.3-13.6)</td>
</tr>
<tr>
<td>Residence</td>
<td>Northern</td>
<td>36</td>
<td>24</td>
<td>36</td>
<td>4.2</td>
<td>(1.3-14.2)</td>
</tr>
<tr>
<td></td>
<td>Eastern</td>
<td>12</td>
<td>4</td>
<td>12</td>
<td>2.0</td>
<td>(0.1-34.6)</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>46</td>
<td>44</td>
<td>46</td>
<td>0.0</td>
<td>(3.4-25.6)</td>
</tr>
<tr>
<td></td>
<td>West</td>
<td>9</td>
<td>15</td>
<td>9</td>
<td>3.3</td>
<td>(0.9-11.0)</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>12</td>
<td>4</td>
<td>12</td>
<td>0.0</td>
<td>(0.0-6.8)</td>
</tr>
<tr>
<td></td>
<td>M-N</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0.5</td>
<td>(3.3-13.1)</td>
</tr>
<tr>
<td>Tribe</td>
<td>Mlchion</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>6.0</td>
<td>(0.6-50.0)</td>
</tr>
<tr>
<td></td>
<td>Northern</td>
<td>87</td>
<td>58</td>
<td>87</td>
<td>5.5</td>
<td>(2.5-12.2)</td>
</tr>
<tr>
<td></td>
<td>Column</td>
<td>13</td>
<td>30</td>
<td>13</td>
<td>3.4</td>
<td>(1.5-85.3)</td>
</tr>
<tr>
<td></td>
<td>Sudanie</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2.3</td>
<td>(3.1-88.5)</td>
</tr>
<tr>
<td></td>
<td>M-N</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5.9</td>
<td>(3.0-11.5)</td>
</tr>
</tbody>
</table>

Smoking
Table 13 Interaction Between Use of Tobacco and Other Exposures Relative Risk and Confidence Intervals*

<table>
<thead>
<tr>
<th>Tobacco</th>
<th>Cases</th>
<th>Controls</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-users</td>
<td>32</td>
<td>65</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Users</td>
<td>62</td>
<td>29</td>
<td>5.0</td>
<td>(2.3 - 15.3)</td>
</tr>
<tr>
<td>Seating</td>
<td>43</td>
<td>32</td>
<td>1.0</td>
<td>(0.24 - 4.4)</td>
</tr>
<tr>
<td>Alcohol users</td>
<td>43</td>
<td>29</td>
<td>4.3</td>
<td>(1.0 - 22.9)</td>
</tr>
<tr>
<td>Tobacco*smoking</td>
<td></td>
<td></td>
<td>0.8</td>
<td>(0.15 - 5.2)</td>
</tr>
<tr>
<td>Tobacco*alcohol</td>
<td></td>
<td></td>
<td>0.3</td>
<td>(0.15 - 5.8)</td>
</tr>
</tbody>
</table>

All adjusted for age, sex, tribe, residence, and included interaction terms for seating and alcohol consumption.
Attributable-risk were calculated as above. Among the controls 30.9% used loombak. Applying the adjusted RR of 5.3 after controlling simultaneously for multiple variables and using interaction terms. Estimates of attributable risk in loombak users who didn't smoke or use alcohol was 65.2%. Thus about two-thirds of cancers of lip, buccal and floor of mouth were attributed to use of loombak.
4.7 Discussion

4.7.1 Case control

The cases for this study were selected from among hospital admissions not from a defined population. Therefore one of the two control groups was selected from the same hospital as the cases, to control for unknown factors related to hospitalisation. The two case groups were chosen so as to distinguish between the effects of direct contact of toombak and distant or no contact. The second control group, volunteers who came for health education, provided an independent estimate of use of toombak in people without oral cancer. Although neither of the control groups was ideal, the consistency of the results based on the two different control groups is supportive of a causal association. A consistent finding of studies on the association of smokeless tobacco and oral cancer is that the cancer more frequently develops at the site of application of the tobacco quid

The findings of the present study provide strong evidence of an etiological association between use of toombak and oral cancer in Sudan. The relative risk is most strongly increased for cancers at sites that came in direct contact with the tobacco quid, and risk is particularly high among long term users. Supporting evidence for excess risk of oral cancer among snuff users in USA, Wann et al (1981), Surgeon General, USA (1986) and Europe IARC (1985), Surgeon General (1986) and tobacco mixed with tobacco (Khaini) Asia (Orr et al 1933), Findborg et al (1965; 1980), Hiyama (1986), and Gupta et al (1989) has been reported.
In various parts of the world, intraoral sites reported to have a direct contact with tobacco quid are buccal mucous, labial mucous, gingiva, floor of the mouth, and lateral aspects of the tongue (William et al. 1977, Juwassilla et al. 1970, Juwassilla et al. 1971). Among the Sudanese the gingivolabial and gingivolabial folds and floor of the mouth are sites in direct contact with the quid (El-beshir et al. 1989). The highest relative risk in found to be associated with these sites in this report. The only descriptive study from the Sudan which examined oral cancer in relation to use of toombak at these sites in some detail was by El-beshir et al. (1989). They reported that there was a close association of the tumor site with the area in which the snuff was habitually placed the floor of mouth, lower gingivolabial sulcus, and gingivolabial sulcus.

It has been observed that at these sites maximum contact with the ingredients of the quid could be maintained by the buccal mucous membranes (William et al. 1977, Juwassilla et al. 1970), Juwassilla et al. (1971). Similarly Sudanese toombak users retain the quid either in the gingivolabial sulcus or gingivolabial sulcus for periods ranging from a few minutes to several hours. While they continuously spat the undissolved particles, the quid got mixed with new saliva to offer continuous flow of new extract from the grid. Edentulous patients can only retain the quid in the floor of the mouth anteriorly or laterally, and the quid is held by the ventral and lateral surfaces of the tongue. It has been found that the floor of mouth was the most frequently involved (39%) among toombak users in Sudan (El-beshir 1989).
In comparison the lower risk associated with use of smokeless tobacco for cancers of the palate, alveoli, and anterior two thirds of the tongue is argued to be due to the fact that contact with the liquid extract of the ingredients can be maintained only for a short period. Lower relative risks are also found at sites at a distance from the tobacco quid such as the oropharynx and oesophagus; this is explained by the fact that these sites do not come in contact with the extract (Juwara Salia et al. 1970), Juwara Salia et al. (1971).

The association between loombak and oral cancer in Sudan is further strengthened by factors related to demographic characteristics, of the population.

The increased relative risk for oral cancer seen among older individuals in this study is possibly due to the fact that older individuals are likely to have used loombak for a longer duration, tend to keep the quid at same site, and use larger quids.

The similarity between the geographic and tribal distribution suggest that major tribal groups have retained their cultural habits in the original areas of residence.

The most important factor for the high relative risk of oral cancer among the gudlein, sudein, hija, and juhiana tribes, is most likely the extensive loombak habit in males and females among these tribes.

Snuff used in western Europe and north America has been established to contain human carcinogens such as volatile
aldehydes, polynuclear aromatic hydrocarbons (PAH), polonium-210 and volatile N-nitrosamines. NNN, NNK, NAB and NAT which have been found to be associated with increased risk for oral cancer IARC (1985), The Surgeon general, USA (1986).

Recently *toombak* and saliva of *toombak* users in Sudan were found to contain unusually high levels of tobacco specific nitrosamines (TSN), NNN and NRK Idris et al (1991).

It was expected that the finding of unusually high TSN would correlate with unusually high relative risk among Sudanese. However, the relative risk of 5-11 among *toombak* users, reported by this study was similar to those reported from many parts of the world. This raises the question of whether any bias in recording the information could have contributed to the difference between cases and controls. The comparability of cases and controls has already been discussed. However, as the data was obtained from hospital records, it is likely that the exposure under study has been underestimated among the cases and the hospital controls. The cultural and religious practices of Sudanese people may have some influence in modifying the effects of carcinogenicity. The older literature, has frequently stated that oral cancer is associated with poor oral hygiene and oral betel (50). About 80% of Sudanese people are Muslims. According to Islam, a Muslim should brush his teeth or at least rinse his mouth three times, before each of the five prayers. The five prayers are scheduled to be practised throughout the day, daily from before sun rise to before going to bed at night daily from seven years of age.
till death. Furthermore during the holy month of fasting (Ramadan) toombak use is prohibited from sun rise to sun set. Because of the hot dry weather, Sudanese drink water several times a day the toombak user naturally has to wash his mouth before drinking or eating. They also habitually wash their mouth when they finish dipping the quid and spit the bland product. This repeated process of washing the mouth many times a day would probably help washing away most of the nitrosoamines. Analysis of the saliva of Sudanese toombak users detected very little nitrosoamines before dipping the quid but high concentration, after end of toombak dipping Idriz et al (1991). Another factor could be provided by the fact that due to the burning effects of natron (sodium carbonate and bicarbonate El-beshir et al (1989), toombak users frequently changed the site of placement of the quid within the gingivalabial or gingivobuccal sulcus. Therefore each specific intra-oral site would have less frequency of direct contact with the quid. Young individuals generally use small quids with more frequent changes of the site of placement compared to older individuals.

The low relative risk found in individuals who smoke and use toombak compared with non-smokers who use snuff has been reported Winn et al (1981) Jwasswilla et al (1970). It has been argued that person with both habits would have shorter periods of exposure than do those with only one habit Winn et al (1981). The carcinogens of inhaled tobacco fumes during smoking produce their effects posteriorly on the oropharynx and soft palate, rather than anteriorly in the oral cavity where the tissues are not in direct contact with stream of carcinogens from smoke Jwasswilla et al (1970).
Dependence on *toombak* habit is probably much stronger than cigarette smoking. Since *toombak* contains a sodium carbonate and bicarbonate of pH 8.8 and a tobacco with an unusually high nicotine Idris et al (1981). In addition to buffering property of saliva. It has been reported that in an alkaline pH nicotine is completely unprotonated and its rate of absorption is increased (Brunneman and Hoffmann, 1974). Nicotine will reach the central nervous system very quickly, from the oral cavity (Brunneman et al 1985).

In conclusion this paper draws attention to data relating to the association of *toombak* use among the Sudanese and oral squamous cell carcinoma. However, it should not be considered an exhaustive study, taking into consideration the limitation of the hospital questionnaire which did not enquire about many details relating to the habit of *toombak*, smoking and alcohol use, desirable for analysis. Furthermore some of the information recorded was either imprecise or missing. Our main goal is to present major findings, with respect to *toombak* and its association with oral cancer. Further detailed studies are needed to provide comprehensive information about the factors related to the risk associated with use of *toombak* in Sudan. We hope that the results presented here would provide stimulus for future studies.
4.7.2-Case control 2

The present study examines the risk factors for buccal cavity, floor of mouth and labial mucosa in more details than the above study. It is believed that epidemiological evidence is much stronger. Daily frequency, long term exposures, duration of the quid in the mouth, smoking of cigarettes and consumption of alcohol were studied. Information was obtained by direct interviews of cases and controls using standard questionnaire and trained interviewers.

The finding of excess risk for oral cancer among toombak users confirms earlier observations. The increased risk was 5.9. Among long term users the risk was nearly twenty-fold. The relative was highly increased with increase in the frequency of use per day and duration of retention of toombak in the mouth and at least 80 per cent of these cancers are attributed to use of toombak. The relative risk estimates are higher in the present study than our previous case control study (Ibris et al 1991). We believe that the elevated RR in this study was obtained because of a better ascertainment of exposure by interviewing of cases and controls in more details.

The findings were similar to those reported among snuff users from the USA (Winn 1981) Sweden (Axell, 1975) chewing of tobacco containing products in South and South East Asia (Pindborg, 1981). The sites studied were those in which tobacco was habitually placed.
In the Snuff used in Western Europe and North America, several probable carcinogens have been established, IARC (1985) including N-nitrosamines, volatile aldehydes, polynuclear aromatic hydrocarbons (PAH), and polonium-210. Of these, N-nitrosamines have been reported to be at the highest concentrations (ug/d) (Hoffman & Adams 1981; Hoffman et al 1988).

Recent studies analyzed Sudanese toombak (Idris et al 1991), and saliva of toombak users (Idris et al 1981) to determine the levels of carcinogenic tobacco specific nitrosamines (TSNA). Unusually high levels of TSNA were found.

Exposure to tobacco specific nitrosamines (TSNA) has been measured in the saliva of 12 users of Sudanese oral snuff (toombak). Nine out of 10 subjects had detectable saliva levels of total TSNA before chewing (0.01-1.0), and immediately following chewing (0.1-2.0) ug/ml.

After controlling for demographic variables the relative risk was not significantly altered. Variables that indicated the degree of exposure to toombak consistently showed increase in the RR as the exposure increased. A stepwise logistic regression analysis indicated independent effects of toombak, smoking of cigarettes and alcohol. The relative risk was even higher among toombak users who did not smoke or use alcohol.

The risk for oral cancer didn’t increase among toombak users who were also cigarette smokers. The proportion of
cigarette smokers among cases and controls were similar. Earlier studies from India have shown that the proportion of smokers was reportedly higher among controls as compared to oral cancer cases. Saighi et al 1955, Shanta & Krishnamourthy, 1969, Shanta & Krishnamourthy, 1963. In 1972, Winn et al carried a case control study and found that most the cases who dipped snuff did not smoke cigarettes or consume alcohol, and the risk for oral cancer was not high among those dipped snuff and smoked cigarettes.

Despite the possibility of under reporting, alcohol was observed to be an independent risk factor after adjustment for other risk factors, relative risk is 4.9 (95 per cent confidence limits 1.0,22.9). Thus the effects of alcohol on risk for oral cancer may be stronger than that observed. Alcohol has not been reported as risk factor for oral cancer in Sudan before. However, alcohol was reported as risk factor among smokers in the USA, Winn et al 1981, Rothman and Keller 1972.

Even though alcohol has emerged has as a risk factor in this study, the results need confirmation by future studies before accepting them.

Despite the limitation of hospital based survey, the Khartoum Teaching Dental Hospital and the Radiotherapy and Isotopes Center Khartoum, are the only centers for treatment of patients having oral cancer, it is expected that maximum number of cases would be identified. Assuming that the cases and controls were truly representative of the people of the Sudan, the results implicates toonbak as responsible for high rate of oral cancer in the Sudan. Kosa (1980)
using data from period (1968-1977) and period (1978-1980) estimated a high frequency of oral cancer, 8.5%, data from RICK. The relative frequencies of oral cancer was 7% among females and 12.9% among males. Biryana (1987) found that Sudan was in the lead for cancer of the buccal mucosa in Africa.

Parkin (1984) compiled the age standardized frequencies of cancer in developing countries. The figures show that the highest rates in Africa were in Sudan, Rwanda, Angola and Tunisia. There was a high frequency of oral cancer in all of these countries.

Idris et al (1991) using, data from 1915 cases of oral neoplasms occurring in a 15-year period, revealed that the frequency of oral neoplasms is relatively high when compared with neighboring countries and accounts for about 7.5% of all neoplasms of the pathological sites studied.
Chapter Five

Toxicity and Carcinogenicity of Toombak
5.1-Introduction

The oral use of tobacco has been associated with human oral cancer by epidemiological studies in North America and Western Europe and on betel quid users in Asia (IARC 1985). Although oral use of tobacco is prevalent in Africa and the Eastern Mediterranean, only a few studies are available on the preparation and use of smokeless tobacco in these regions El-Akad et al (1986), Obet, I.S (1980), Salem et al (1984), Stirling et al (1979), Talor, (1963), Yousef et al (1983). Studies on oral users in these regions frequently report information on customs and habits of usage Stirling et al (1979), Hickey (1959) and public health implications of oral tobacco use have not been described. In addition to the previous clinical studies, it was planned that tests of toombak for probable carcinogens to be undertaken.

This study was undertaken to investigate the exposure of toombak users in Sudan to permit an evaluation of the role of these nitroamines in the etiology of oral cancer. Toombak was collected from toombak shops and saliva samples, collected from toombak users before, during and after dipping, were analyzed for TSNA and nicotine. Chemical analyses were done in France with the collaboration of IARC.
5.2 Review of the Literature

In the Snuff used in Western Europe and North America, several established and probable carcinogens have been quantified, including N-nitrosamines, (volatile and non-volatile), volatile aldehydes, polynuclear aromatic hydrocarbons (PAH), and polonium-210. Of these, N-nitrosamines have been reported to be present at the highest concentrations (µg/g) (Hoffman & Adams 1981; Hoffman et al. 1986) and are considered to be among the likely causative agents for cancer of the upper alimentary tract, lung and urinary bladder among tobacco users (Bartosh & Mantzorn, 1984; Hoffman & Hecht, 1985; Hecht & Hoffman, 1988). Of the 32 N-nitroso compounds that have been identified in smokeless tobacco products, tobacco-specific nitrosamines (TSNA) derived from Nicotiana Alkaloids contribute 70-80% of the total amount (Tricker & Freundmann 1990). Among the TSNA, N-nitrosornornicotine (NNN) and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butamone (NNK), are potent carcinogens in various animal species; N-nitrosomabasine (NAB) has been reported to be a weak carcinogen (Hoffman and Hecht 1985), with a limited evidence for its carcinogenicity in experimental animals (IARC, 1985).
5.3-Materials and Methods

Samples: Toombak samples prepared from N.Rustica (Chap1) were obtained from local shops in Khartoum, Sudan. Samples were obtained from five different quality levels (see Table 14) based essentially on price were obtained from each supplier. Two were obtained from N.Glaucous (Chap1) which is used only in a small region in the South of Sudan.

Saliva samples from toombak users (Males aged 10-70 years) were collected before, during, and after toombak use at Soba University Hospital, Khartoum (15-16 Cont.). After adding sodium hydroxide (1 N NaOH 0.1 vol) to stop nitration immediately after collection, all samples were frozen and transported in a dry ice to Lyon, France, were stored in -20°C prior to analysis.

Chemicals

MNN and MNE were purchased from Chemsyn laboratory, Leneza Kansas, USA. NAT and NAB were a generous gift from Dr. Hoffmann, Naylor Dunn Institute for Disease Prevention. American Health Foundation Valhalla, NY, USA. N-Nitroso-N-butylnaphthylamine (NBNbaA), used as internal standard, was synthesized as reported (Nair et al 1985). Nicotine was obtained from Sigma, St. Louis, MO, USA and 1-methylpyridine was purchased from Fluka AG, Buchs, Switzerland. The purity of the chemicals used as standard was verified by TLC and gas chromatography (GC) with flame ionization detection.

Extraction and analysis of samples:

N-nitrosamines and nicotine were extracted from toombak and saliva samples by a procedure described.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Quarts</th>
<th>Minerals</th>
<th>Nitrate</th>
<th>Nitrite</th>
<th>Cyanide</th>
<th>Total Toxic Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/g dry wt)</td>
<td>(mg/g dry wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg/g dry wt</td>
</tr>
<tr>
<td>46</td>
<td>20.6</td>
<td>12.5</td>
<td>3.13</td>
<td>0.02</td>
<td>0.21</td>
<td>3.25</td>
</tr>
<tr>
<td>47</td>
<td>7.2</td>
<td>187.4</td>
<td>1.15</td>
<td>0.29</td>
<td>1.87</td>
<td>3.34</td>
</tr>
<tr>
<td>48</td>
<td>6.4</td>
<td>35.3</td>
<td>1.12</td>
<td>0.15</td>
<td>1.05</td>
<td>3.32</td>
</tr>
<tr>
<td>49</td>
<td>17.2</td>
<td>37.2</td>
<td>0.13</td>
<td>0.05</td>
<td>0.07</td>
<td>0.34</td>
</tr>
<tr>
<td>50</td>
<td>65.3</td>
<td>35.3</td>
<td>1.44</td>
<td>0.08</td>
<td>0.07</td>
<td>2.09</td>
</tr>
<tr>
<td>51</td>
<td>153.3</td>
<td>35.3</td>
<td>2.15</td>
<td>0.14</td>
<td>0.71</td>
<td>3.79</td>
</tr>
<tr>
<td>52</td>
<td>14.6</td>
<td>35.2</td>
<td>0.18</td>
<td>0.14</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>53</td>
<td>15.1</td>
<td>26.3</td>
<td>0.12</td>
<td>0.13</td>
<td>0.15</td>
<td>0.33</td>
</tr>
<tr>
<td>54</td>
<td>15.4</td>
<td>26.4</td>
<td>0.08</td>
<td>0.13</td>
<td>0.15</td>
<td>0.32</td>
</tr>
<tr>
<td>55</td>
<td>20.9</td>
<td>26.2</td>
<td>0.00</td>
<td>0.03</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>56</td>
<td>17.5</td>
<td>4.4</td>
<td>0.12</td>
<td>0.03</td>
<td>0.15</td>
<td>0.43</td>
</tr>
<tr>
<td>57</td>
<td>11.9</td>
<td>25.5</td>
<td>0.09</td>
<td>0.02</td>
<td>0.02</td>
<td>0.17</td>
</tr>
<tr>
<td>58</td>
<td>62.6</td>
<td>39.5</td>
<td>2.05</td>
<td>0.05</td>
<td>0.04</td>
<td>2.14</td>
</tr>
<tr>
<td>59</td>
<td>6.2</td>
<td>23.6</td>
<td>0.32</td>
<td>0.08</td>
<td>0.02</td>
<td>0.42</td>
</tr>
<tr>
<td>60</td>
<td>6.1</td>
<td>18.3</td>
<td>0.48</td>
<td>0.02</td>
<td>0.02</td>
<td>0.52</td>
</tr>
<tr>
<td>61</td>
<td>6.5</td>
<td>28.7</td>
<td>1.02</td>
<td>0.15</td>
<td>0.23</td>
<td>2.39</td>
</tr>
<tr>
<td>62</td>
<td>15.5</td>
<td>37.9</td>
<td>1.16</td>
<td>0.07</td>
<td>0.17</td>
<td>2.37</td>
</tr>
<tr>
<td>63</td>
<td>11.5</td>
<td>48.6</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.17</td>
</tr>
<tr>
<td>64</td>
<td>10.8</td>
<td>28.8</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.17</td>
</tr>
<tr>
<td>65</td>
<td>1.1</td>
<td>0.5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>2.49</td>
</tr>
</tbody>
</table>

**Note:** Not detected
earlier (Nair et al. 1985; Oehme et al. 1985). NBuBzA and isocinchonidine were added as internal standards to the samples prior to extraction. The extracts were analyzed for TSNA on a Perkin-Elmer (Model Sigma 25) GC coupled to a thermal energy analyzer (TEA, model 543, Thermodics Inc., USA). Separation was achieved on a 2 m x 3 mm i.d glass column packed with 10% UCW 882 on Chromosorb WHP 80/100 mesh (Supelco, Inc., Bellefonte, PA, USA). After 12 min at an initial temperature of 220°C, the column temperature was raised at 10°C/min to 250°C where it was held for five minutes. Nicotine was measured by a method published previously involving capillary GC separation and a nitrogen phosphorous detection (Nair et al. 1985).
5.4-Results

Toombak samples

A typical chromatogram for the GC/TIA analysis of the four TSNA is shown in Figure 11. Table 14 reports the levels for four TSNA in ready-to-use toombak from Khartoum. Moisture content ranged from 6-60% and nicotine content from 8-102 mg/g dry weight. Unexpectedly high levels of NNK (up to 7.87 mg/g dry weight) and NNN (up to 3.08 mg/kg dry weight) were found in these samples.

Fig 12 shows the levels of specific TSNA expressed as a percentage of total TSNA. In this analysis it was found that NNK represented at least 50% of all TSNA in all of the samples except A4 (38%) and D17 (38%). NNN represented 22-53% of all TSNA. In samples A4 and D17, samples with the highest percentage of total TSNA as NNK, NNK represented 52% and 53%, respectively. NAT and NAB represented a small percentage of all TSNA, ranging from 1-7%. Samples with the highest total content of TSNA were samples A1 (31.24 mg/g dry wt), A3 (5.45) and D15 (5.99). The sample with the lowest value for TSNA was sample A4 (1.59 mg/g dry wt). The moisture content of the toombak samples ranged from 5.4-62.62%. Nicotine content ranged from 8.36-102.38 mg/g dry weight. Samples with highest nicotine levels, A2 (102.38 mg/g dry wt) and A3 (73.30) also had high total TSNA levels (31.24 and 5.45 mg/g dry wt, respectively). However, this association didn't hold across all samples. This is in agreement with previous studies where positive correlations of nicotine and TSNA content have not been seen (Brummezzann et al., 1985; Andersen and Kemp, 1985).
FIGURE 3
Percentage of TSMA in Combed...
A chromatogram for the GC/TEA analysis of the four TSNA A chromatograms for the GC/TEA analysis of the four TSNA and the internal standard in sample P10.

Detector Response

4.10 NS-BzA
8.00 NAT
6.88 NNK
8.62 NAr
11.83 NNK

Time (min)
Saliva samples

Figure 13 shows a typical GC-TEA chromatogram for the extract of saliva for a Sudanese snuff diper. In addition to NNN, NAT, NAB, Figure shows a typical GC-TEA chromatogram for the extract of saliva for a Sudanese snuff diper. In addition to NNN, NAT, NAB, and NNK, identified by their relative GC retention times and confirmed by GC/MS. Two tea positive peaks eluting after NNK were observed (peak 1, 12.5 min and Peak 2, 16.8 min). To identify these peaks, fresh extract of saliva were prepared and analyzed by GC/MS. The mass spectrum for peak 1 (Fig 14), with major ions at m/z 150 (base peak) 132, 130, 117, 119, 92, and 78, is similar to that reported for iso-NNAL (Branneman, et al 1981). As both compounds are destroyed during the storage of extract in dichloromethane, it was important to carry out GC/MS immediately after extraction. Quantitation of NNAL and iso-NNAL in iso-NNAL in saliva was done by comparison with internal standard.

Table (15 cont.) summarizes the levels of TSNA in the saliva of snuff dippers before, during and after diping the snuff. Nine out of 10 subjects analyzed already had detectable amounts of TSNA present in their saliva collected before dipping (range 0.011-0.097 ug total TSNA/ml). Levels of individual TSNA in these samples ranged from 0.001 to 0.56 ug/ml (NNN) (detected in 3 out of 10 samples) and 0.002-0.036 ug/ml (NNK) (detected in 3 out of 10 samples).

During dipping, TSNA concentration up to microgram milliliter levels were observed for individual nitrosamines (NNN, 20.19 ug/ml; NNK, 6.7 ug/ml) and for total TSNA (0.7-30.6 ug/ml). NNN represented 77±10 % of the total TSNA.
detected in saliva during usage, whereas NAB and NNK represented 6 ± 1 % and 10 ± 10 % (1 detected in one sample), respectively. In addition to these TSNA commonly found in the saliva of snuff dippers NNal and iso-NNAL, which were identified in saliva for the first time, represented 7 ± 5 % (not detected in 1 of 12 samples) and 2 ± 0.5 % (not detected in 4 out of 12 samples), respectively, of the detectable TSNA in saliva during dipping. In contrast only 2 of the 12 subjects had measurable levels of NAT.

Nine out of 10 subjects had detectable TSNA levels even after finishing dipping (NNN, 0.018–1.8 μg/ml, not detected in two samples; NNk, 0.014–0.34 μg/ml, not detected in 4 samples). Total TSNA levels in these samples ranged from 0.1–2.6 μg/ml (not detected in 1 sample).
Table 15

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>Height</th>
<th>Weight</th>
<th>BMI</th>
<th>Body Fat</th>
<th>Resting Metabolism</th>
<th>Total Energy Expenditure</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>Male</td>
<td>178</td>
<td>65</td>
<td>25</td>
<td>34%</td>
<td>2496</td>
<td>1100</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>Male</td>
<td>180</td>
<td>70</td>
<td>28</td>
<td>23%</td>
<td>2736</td>
<td>1300</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>Male</td>
<td>182</td>
<td>75</td>
<td>30</td>
<td>19%</td>
<td>2980</td>
<td>1500</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>Male</td>
<td>184</td>
<td>80</td>
<td>32</td>
<td>15%</td>
<td>3232</td>
<td>1700</td>
<td>40</td>
</tr>
</tbody>
</table>

Note: BMI = Body Mass Index; Resting Metabolism = Metabolic Rate at Rest; Total Energy Expenditure = Total Energy Expenditure per Day.
Table 15 (cont.)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age (yr)</th>
<th>Duration (in months)</th>
<th>PERG</th>
<th>RAT</th>
<th>GUS</th>
<th>HAM</th>
<th>U4-HAM</th>
<th>U4-MAL</th>
<th>MAL</th>
<th>Total ERG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>7</td>
<td>0</td>
<td>0.5</td>
<td>0.9</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
<td>0.3</td>
<td>0.1</td>
<td>1.6</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>0</td>
<td>0.6</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
<td>2.1</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>0</td>
<td>0.4</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>0</td>
<td>0.7</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*not observed*  
*Sample not available for analysis*
Figure 11 Mass spectrum of unidentified peak 1 from saliva samples from toothbrush user. The spectrum peak 1 compares closely with that reported for 4-(methylamino)-4-(pyridyl peak)-1-butene (iso-NAAL).
Fig. 8. Representative GC-TEA chromatogram of TSNA extracted from the saliva of a Sedanese snuff-dipper. In addition to identified TSNA, two TSA-positive peaks eluted after NNK (NBuBzA, internal standard).
4.5 Discussion

**Toombak**

This the first analysis on levels of TSNA in Sudanese *toombak*. Among the types of unprocessed tobacco analyzed from various regions of the world, snuff (used for dipping) has been reported to have the highest levels of carcinogenic TSNA with maximum levels of NNK and NNN reported to be 0.154 mg/g and 0.014 mg/g, respectively (Brunnemann et al. 1996). It has been noted that snuff has higher levels of nitrosamines than any other consumer product, exceeding other levels by over two orders of magnitude (Brunnemann et al. 1996). In comparison, Sudanese *toombak* is even more highly contaminated with TSNA concentration ranging up to 150 mg/g levels. Especially noteworthy are the high levels of NNK, the most potent carcinogenic nitrosamine amongst TSNA. Levels of NNK in snuff samples have been reported to be approximately 10-20% of those of NNN (Brunnemann et al. 1987; Hoffman et al. 1986; Tricker & Frausmann, 1989). However, in Sudanese samples of *toombak*, NNK levels were 150-270% of NNN.

The high levels of TSNA in *toombak* could be partly due to the use of *Rustica* in its preparation. NNK and NNN levels in *Rustica* have been reported to be much higher as compared to N. *tobacum* (Shide et al. 1987). The results of preliminary findings using *toombak* prepared with N. *glauca* rather than N. *Rustica* agree with these suggestions. Another factor may be the treatment with atomizer together with subsequent storage before use. One of the N. *glauca* samples analyzed, which was processed without atomizer, had much lower levels of TSNA than samples which was processed...
without atrau (Table 14). This is interesting because atrau is a chemical substance and one could postulate that such raising the pH would inhibit nitrosamine formation. However, there may be other differences in the way two types of tobacco were processed; for example, it is common for the N. glauca to be left to air dry after processing while toombak prepared from N. rustica is used wet. In agreement with other studies, neither nicotine content nor the moisture content correlated with TSNA levels in N. rustica toombak.

The carcinogenicity of NNN and NNK in rodents is well established (Hoffmann & Recht, 1985). NAB is reported to be a weak oesophageal carcinogen in rats where as NNAL induced lung tumours in A/J mice (Escamay et al.). The occurrence of iso-NNAL in tobacco products has been demonstrated only recently and not much is known about its genotoxic effects except in primary rat hepatocytes (Brunnemann).

On the basis of an NNN and NNK content > 67 μg/g tobacco, it has been estimated that a snuff user in the USA is exposed to ~ 0.7 nmol/kg b.w of NNN and NNK in 40 years of snuff dipping, which is very close to the dose (1.8 nmol/kg b.w.) sufficient to induce a significant incidence of oral tumours in rats (Recht & Hoffmann, 1988). If a similar extrapolation were done for Sudanese toombak user, he would be exposed to an equivalent amount of (1.8 nmol/kg) alone in less than 6 years, even if he consumes half the amount of snuff his USA counterpart (mean NNK level) in Sudanese toombak = 2.2 μg/g).
Saliva

This the first report on levels of TSNA in the saliva of toshbaq users from the Sudan. Levels of TSNA in the saliva of subjects using smokeless tobacco Nair et al (1985), Hoffmann et al (1991), Bhide et al (1986), Braunemann, et al (1987), Osterdahl et al (1984) Sipahimali (1984), Wenke et al (1984). Wenke (1984). (see table) have been reported to be in order of a few hundred nanograms per millilitre. However, in this study it was found that the levels of NNK and NNk in the saliva of Sudanese snuff dippers (μg/ml) at least 10 to 100 times greater than previously reported.

Further, the presence of NNAL and iso-NNAL in the saliva of smokeless tobacco users was demonstrated for the first time. Another important observation is the presence of detectable levels of TSNA in the saliva of subjects before and after snuff-dipping implying a prolonged exposure of TSNA to the oral mucosa. Such long-lasting TSNA levels in saliva do not appear to have been reported before.


In the above studies it was found that toombak contains levels of NNN (0.50-3.1 mg/g toombak dry wt.) and NNK (0.629-7 mg/g toombak dry wt.) that are 20 and 580 times higher,
respectively, than for any previously reported snuff samples Idris et al. (1991). The results presented here on saliva levels are consistent with these previous findings. On the basis of an NNK and NNal content of 67-ug/g tobacco, it has been estimated that, a snuff user in the USA is exposed to -0.7 mmol/kg bw of NNK and NNal in 40 years of snuff dipping, which is very close to the dose (1.6 mmol/kg bw) sufficient to induce significant incidence of oral tumours rate Hecht & Hoffmann (1989). If a similar extrapolation were made for user of toombak (Mean NNK level 2.2 pg/g), he would be exposed to an equivalent amount (1.6 mmol/kg) of NNK alone in less than six years, if consumed only half the amount of snuff of his US counterpart. Among the 12 subjects from whom saliva samples were collected for this study, 10 had used snuff for more than seven years (See table (22 cont)

In conclusion, these studies had shown that toombak as form of smokeless tobacco had a highest yet reported TSNA levels. The carcinogenicity of TSNA for humans were unequivocally established.

Extensive clinical and epidemiological data are now available for the Sudaneses population. El-beshir et al. (1998, Chap2-Chap4) and all concluded that the risk for oral cancer was several times higher among toombak users. Thus, toombak-dipping in the Sudan should be considered as a major risk factor for oral cancer and other tobacco related neoplastic diseases. Snuff dipping has been strongly associated with cancer of the oral cavity Olleslein (1991), Gou et al (1991), Winn el al (1981), US Department of Health and Hum Winn et al. (1991), US Department of Health
and Human Services (1986). It has been shown in Denmark and Sweden that TSNA levels can be reduced by changing tobacco species or modifying the fermentation and processing of tobacco Osteråhl & Slørøg (1984), Brunnermann et al (1985), Appel (1981) and similar modification of Sudanese tobacco to reduce TSNA merits further investigation. However, sufficient evidence for promoting public health officials to educate toombak users about risks of dipping toombak and to promote cessation of tobacco use.
General Discussion & Conclusions
The evidence accumulated from the studies of toombak, the descriptive epidemiology of patients with oral cancer, the two analytical epidemiological studies together with the experimental investigation of presence of carcinogens in toombak and saliva of toombak users strongly indicated that the use of toombak can cause oral cancer.

Studies of toombak plant disclosed for the first time that the toombak plant used for manufacture of toombak product is of the species within the genus Nicotiana (Solanaceae), N. Rustica. Basic information about the characteristics of the toombak product has been provided.

The descriptive study showed that the rates of exposure to toombak and oral cancer were high among the Sudanese population. Based on the descriptive study & hypothesis was generated that the high rate of use of toombak may be the cause for the high rate of oral cancer in the Sudan.

Sufficient evidence for causal relationship between the exposure to toombak and oral squamous cell carcinoma was derived from two analytical epidemiological studies. The risk was several times higher among toombak users and that among long term users the risk was even higher. The cancer developed at sites of placement of the toombak quid, users who retained the toombak quid for longer duration had much higher rates than users of toombak that did not.

This conclusion was consistent with the judgement of the expert committee of the IARC (1985), stated that "Evidence for carcinogenicity in humans can be derived from case reports, descriptive epidemiology and analytical
epidemiology". The expert committee of IARC, interpreted an analytical epidemiology as implying causality to a greater or lesser extent if the study provided sufficient evidence that a causal relationship between exposure and cancer is credible. Bias, chance, interaction or confounding factors were adequately excluded. Similarly, Breslow and Day (1980) stated that "the interpretation of a study involves evaluating the likelihood that the results reflect one or more biases in design or conduct, the role of confounding, the role of chance or the role of causality".

The limitations and strengths of the above epidemiological studies were discussed in details (Chap2-chap4). There was no identifiable positive bias and confounding factors were considered. The causal association was unlikely to be due to chance.

Although a single epidemiological study may be strong indicative of a cause-effect relationship, the most convincing evidence of causality comes when several independent studies done under different circumstances result in positive findings IARC (1985). This assertion by the IARC is consistent with the conclusion derived from the above five independent studies. They were comprehensive. They included descriptive epidemiological studies which correlated occurrence of cancer in space or time to an exposure and two analytical epidemiological studies of the case control type, which assessed the risk for oral cancer
independently and directly on humans. The findings were consistently positive.

Major strengths of epidemiological studies compared with experimental research is that it applies directly to human beings, there is no species barrier to overcome in attempting to infer how applicable the results are to human beings Brenlow and Day (1980). The same authors further reported that the major strengths of the case control design compared with other types of epidemiological research is its informativeness. Case-control studies can evaluate simultaneously many causal hypothesis, interactions, and confounders.

On the other hand, Brenlow and Day (1980) discussed adequately limitations of case control studies. They permit estimation only of relative disease frequency and that the studies are highly susceptible to biases.

Causality of toosbaks to oropharyngeal cell carcinoma ascertained by the above epidemiological studies were further supported by experimental study of toosbaks and saliva of toosbak users for carcinogenicity (chap5). Such studies revealed that toosbaks and saliva of toosbak users contain potent carcinogens, they included the tobacco-specific nitrosamines (TSNA). In addition, two TSNA, 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanol (NNAL), were found in the saliva of toosbak users for the first time and were confirmed by gas chromatography.
The TSNA were detected in toombak and saliva of toombak users, at levels 10–100 times higher than those found in snuff used in Europe and North America. However, animals exposed to these tobacco-specific nitrosamines, at levels approximating those thought to be accumulated during a human lifetime by daily smokeless tobacco user, have developed excess cancers. (The Surgeon General 1986).
Recommended Future Research
The natural history of squamous cell carcinomas among tobacco containing quid in Asia and snuff users in USA and Europe is well established. Various premalignant lesions associated with initiation and development of squamous cell carcinomas were identified. Such premalignant lesions have not been observed in squamous cell carcinomas associated with use of toombak in the Sudan. Studies from the Sudan on the natural history of oral squamous cell carcinomas among toombak users are lacking. Such studies are necessary to examine if development of squamous cell carcinomas among toombak users in Sudan is preceded by any form of premalignant lesion or condition.

Extensive clinical and epidemiological methods to study whether the unusually high levels of TSNA in toombak and saliva of toombak users are associated with much higher risk for oral cancer as compared to other countries where tobacco contaminated with lower levels of TSNA is used for the same habit.

Adverse health effects smokeless tobacco include effects on the dental tissues such as gingival recession, soft tissue changes at the site of placement of the tobacco, degenerative changes of the salivary gland, cardiovascular and digestive effects, fetal toxicity, and addiction and dependence (IARC 1985; Surgeon General 1986; WHO 1988). There is a need for evaluation of toombak for such adverse health effects.

Design and develop studies for prevention.
Studies on the prevalence of use of toobak, other forms of tobacco, consumption of alcohol and other factors which may have influence in development of these habits. In particular, cultural and socioeconomic factors such as tribal identity, occupation, and geographical area of residence. As knowledge of these factors are imperative for planning prevention programs.

Although cessation of tobacco use will be the only way to totally prevent the exposure to carcinogenic TSNA, the unusually high level of TSNA in toobak could be significantly reduced by choosing tobacco species or by modifying the processing of tobacco, as shown in Denmark and Sweden. Nonetheless, epidemiological studies could be undertaken to investigate aspects of initiation of the habit in children so that preventive measures could be directed to identified target factors.
References


Atkinson L., Chester I.C., Smith, F.G, & Te'n Seldes, R.E.J.: Oral Cancer in New Guinea; Study in demography and etiology Cancer, 17, 1289-1298, 1984

Azell, T. A prevalence study of oral mucosal lesions in an adult Swedish population Odontol. Rev. 27, Suppl.36. 1976


Barry A. Ikhliss, Professor of Botany, Department of Botany, University of Khartoum, personal communication.

Beaumaling N., Keen P., Petering H., Carcinoma of the maxillary antrum and its relationship to trace metal content of snuff, Arch Envi Health vol 23, 1971


tobacco leand processed tobacco leaves from India. Beir.
TobakForsch., 14, 29-32, 1987

Bhide, S.V., Nair, J., Spiegelbalder, B.& Preussmann, R. N- nitrosoamines the saliva of tobacco chewers or masuki users.


Bloodgood, T.C: Cancer of the Tongue; preventable disease
J. A. M. A 77:1381-1397, 1921


Brunnemann, K.D., Prokopczyk, S., Hoffmann, D., Nair, J., Gesham, H., Bartsch, H., Laboratory studies on oral cancer and smokeless tobacco. In: Hoffmann, D. and Harris, C.C. Mechanism in tobacco Carcinogenesis, Benbury Report No. 23. cold Spring Harbour Laboratory USA, pp197-212. 1986


Castonguay A., Peppin, P. & Stoner, G.D.: Lung tumorigenicity of NNK given orally to A/J mice: Its application to

Castonguay, A., Tjia, V., & Hecht, S. S., Tissue Distribution of the Tobacco specific carcinogen 4-(methyl-
nitrosamino)-1-(pyridyl)-1-butanone and its metabolites in F344 rats. Cancer Res., 43, 630-638


Correa, E., Joshi, P.A., Castonguay, A., & Schullier, N.M., The tob: The tobacco-specific nitrosamine 4-
(Caethylamino)-1-(3-pyridyl)-1-butanone in an active
Transplacental carcinogen in Syrian golden hamsters. Cancer Res. 50, 3435-3438


hamster by a single dose of 4-(methylamino)-1-(3-pyridyl)-1-butanone (NNK) and the effects of smoke inhalation. Carcinogenesis, 4, 1287-1290, 1983


Hecht, S.S., & Hoffmann, D., Tobacco specific nitrosamines: an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis, 8, 975-984, 1986


Hidayatalla A., Malik, M.O.A, Malik and El-bald A.E., Osman A.A. and Hutt M.S.: Studies on nasopharyngeal carcinoma in


IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 37, Tobacco habits other than smoking; Betel-Quid and Areca-Nut Chewing; and some related nitrosamines 1985.


Idris A.M. Elsaffa wa saratau el shifa amfira (Arabic) monograph on use of toombak 1982.


Martens, I. Factors associate with cancer of the esophagus, mouth, and pharynx in Puerto Rico J. Nati Cancer Inst., 42, 1069-1094, 11989


Mehta F.S., Pindborg J.J, Gupta P.C., Daftary D.K.: Epidemiological and histological study of oral cancer and
Jeukoplakia Among 50,315 villagers in India. Cancer, 24, 832-840, 1969


Mukhtar B.I. personal communication 1987


Nobri, I.A., Pattern of Surgical diseases in Two separate areas of the Sudan; A study of geographic pathology Int. Path, 9, 52, 1980.


Parkin D.M: Cancer in developing countries: a scientific
publication, of IARC No 75, 1986

Paynester, J.C.: Cancer of the buccal mucosa; clinical study
of 850 cases in Indian patients. Cancer 9:431-435, 1956

Pezack, E.R., Greenberg, B.G., and Brawley, R.W. The effect
of snuff and tobacco on the production of oral carcinoma.

Pindborg J.J. and Poulsen, H.E. Studies in oral leukoplakias
I. the influence of snuff upon the connective tissue of the

Gupta, V.K.: Frequency of oral carcinomas, leukoplakias,
lichen planus, submucous fibrosis and lichen planus in 10,000
Indians in Lucknow, Uttar Pradesh, India. Preliminary
report. J. Dent. Res. 65:615, 1965

Pindborg, J.J., Jølst, O., Bonstrup, G., and Reed-Peterson, B.
Studies in oral leukoplakia: preliminary report on the period
prevalence of malignant transformation in leukoplakia based
on follow-up study of 246 patients. J. Am. Dent. Assoc.
78:767-771, 1968

Pindborg, J.J., Bhonsle, R.B., Murti, P.R., Gupta,
P.C., Dattary, D.K., & Rehns, F.S.: Incidence rate and early
forms of oral submucous fibrosis. Oral. Surg. 50:40-44,
1980


Rivenson, A., Hecht, S.S., & Hoffmann, D.: Carcinogenicity of Tobacco-specific Nitrosoamines (TSNA): The role of the


Wahi P.N., Mital V.P., Lahiri B., Luthra B.K., Seth R.K., and Arora G.D.: Epidemiological study of precancerous lesions


WHO International Classification of Diseases, 8th Revision, Vol 1, Geneva, 1975


Radiation & Isotope Centre Khartoum

Name: ........................................ Reg. No. ........................................

Address: ........................................ ........................................

Date: ........................................ Ref: Doctor & Hosp. ........................................

Age: ........................................ Sex: ........................................ Home: ........................................ Tribe: ........................................

Occupation: ........................................ Habits: ........................................

Duration of Symptoms since first Discovery: ........................................

Duration of Symptoms since first Reporting: ........................................

SYMPTOMS (with dates):

1. ........................................
2. ........................................
3. ........................................
4. ........................................

SIGNS:

1. ........................................
2. ........................................
3. ........................................
4. ........................................

Lymph Nodes:

Distant Metastasis:

X-Ray Findings:

Stage:

General:

Biopsy No.

Final Diagnosis:

Previous Treatment:

........................................
Treatment Plan
Appendix 2

A questionnaire investigating use of loomabak, smoking or cigarettes and use of alcohol

Personal characteristics

1. Sex
2. Age
3. Residence
4. Original resid
5. Tribe
6. Education
7. Address

Loomabak usage:

If Current user go to No 2D
If stopped,
10. Duration of regular use
11. Duration since last use
12. Number of attempts to stop

If Irregular
13. The longest duration of regular use
14. The longest duration of non-use
15. Used loomabak occasionally
16. Used loomabak only if cigarettes are not available
If attempted
17. Why
18. No of attempts
19. Duration of longest attempt
20. Which year did you started using loomabak
21. Duration of loomabak use
23-Quantity used per day (number packages)
24-Frequency of use per day
25-Sleeping with snuff in the mouth
  1-Regular 2-Irregular 3-Never
26-Application of snuff in the mouth?
  1-Lower lip 2-Buccal cavity 3-Below the tongue 4-Upper lip
  5-Nose 6-Any other
27-How did you acquire the habit? from
  1-An elder member of the family 2-more than one member of the family
  3-close friend 4-more than one friend
  5-Work colleagues 6-peer pressure 8-Any other
28-Have you ever used tobacco for relaxing before
requiring the habit?
29-To use tobacco for stressful situations like
examinations?
30-To use tobacco among associates
  1-An elder member of the family 2-more than one member of the family
  3-close friend 4-more than one friend
  5-Work colleagues 6-peer pressure
31-Have you any attempt to quit? if yes
How many times...Duration
32-Smoking cigarettes:
  1-never 2-Current 3-Stopped 4-Irregular 5-Attempted
If Current. go to No. 41
If stopped.
33-Duration of regular smoking
34-Duration since last smoking...
35- Number of attempts to stop............................
If Irregular
36- The longest duration of regular smoking..................
37- The longest duration of non-smoking..................
38- Seeking occasionally..................................
39- Smoking only if toombak is not available.....
If attempted
40- Why.................................................18- No of attempts.............
41- Duration of longest attempt..........................
42- Which year did you started smoking..................
43- Duration of smoking..................................
44- Quantity smoked per day (number packages).........
45- How did you acquired the habit? From 1- An elder member of the family 2- more than one member of the family 3- close friend 4- more than one friend
5- For colleagues 6- Peer pressure 6- Any other
...............................................................
46- Have you ever smoked because of stressful situation like examinations?.................................
47- Seeking among associates 1- An elder member of the family 2- more than one member of the family 3- close friend 4- more than one friend
5- For colleagues 6- Peer pressure
48- Have you made any attempt to stop?.................If you how many times...........
49- have you had any of the followings on your first attempt: 1- Vomiting 2- Nausea 3- Drowsiness 4- Others..........................
50- Alcohol usage
1- Never 2- Current user 3- Stopped 4- Irregular 5- Attempted
If Current go to No 53 If stopped:
51-Duration of regular use of alcohol
52-Duration since last use of alcohol
53-Number of attempts to stop
If Irregular
54-The longest duration of regular use of
55-The longest duration of non-use
56-Use of alcohol occasionally
If attempted
57-Why
If attempted
58-No of attempts
59-Which year did you started use of alcohol
60-Duration of use of alcohol
61-Frequency of use
62-How did you acquired the habit? from 1-member of the family 2-more than one member of the family 3-close friend 4-more than one friend
5-Work colleagues 6-peer pressure 8-Any other
63-Have you ever used because of stressful situation like examinations?
64-Use of alcohol among associates 1-An older member of the family 2-more than one member of the family 3-close friend 4-more than one friend
65-Work colleagues 6-peer pressure
66-Have you made any attempt to quit? if yes How many times