Sudan University of Science and Technology

College of Graduate Studies

Oral Candidiasis in Sudanese Cancer Patients Receiving Different of Treatments for different cancer types

الابيضاض الفموي لدى مرضى السرطان السودانيين الخاضعين لعلاج السرطان بأنواعه المختلفة

A Thesis submitted in partial fulfilment for requirement of M.Sc. degree in Medical Laboratory Science (Microbiology)

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الآيـــــــــــــة

بسم الله الرحمن الرحيم

﴿ إِنَّا فَتَحْنَا لَكَ فَتْحًا مُّبِينًا {1} لِيَغْفِرَ لَكَ اللّهُ مَا تَقَدَّمَ مِن ذَنبِكَ وَمَا تَأَخَّرَ وَيُتِمَّ نِعْمَتَهُ عَلَيْكَ وَيَهْدِيَكَ صِرَاطًا مُّسْتَقِيمًا {2} وَيَنصُرَكَ اللّهُ نَصْرًا عَزِيزًا {3} ﴾

صدق الله العظيم

سورة الفتح : الآيات (1-3)

Dedication

To cancer patients

To my parents

To my sister and Brothers

To my friends

To Teachers and supervisor

Acknowledgement

Thanks almighty ALLAH for helping me, guiding and lighting my thoughts and giving me strength to fight all my fears. Thanks to cancer patients for being cooperative and for being so patience with me, and my condolences goes for those not among us right now and to their families, and my prayers for those fighting and cancer survivors. Great thanks to my family for their support and thanks to supervisor Prof.Yousif Fadlallah Hamed Elnil for his advice and his kindness. Thanks to all staff, laboratory assistant and teachers in Microbiology Department in Sudan University of Science and Technology for their help during work. A great thanks to my job work supervisor in Radio Isotopes –Khartoum Center Mr.Kamal Al Zubir Nogod for his patience and kindness in creating a work schedule suitable with my master programme .Thanks to my colleges and friends for their help and advice.

ABSTRACT

This study was done to detect the prevalence of oral Candidiasis among Sudanese cancer patients with different cancer types who received different cancer treatment in Khartoum oncology hospital which formerly was known as Radio and Isotopes Centre of Khartoum (al Zarra Hospital) which recently named Khartoum Oncology Hospital. .A hundred oral swabs were collected from the patients with different age groups ranging from 2 to 83 old, 66\100 were males and 34\100 were females.The study was carried out in the period from May to July 2015.

The study showed that the distribution of an oral Candidiasis among cancer patients was 40% and age group less than 20 years old were more affected with an oral Candidiasis.

Study showed that males were most affected with an oral Candidiasis than females with 23%.

All samples were first cultured on to Sabouraoud's dextrose agar media which was supplemented with chloramphenicol antimicrobial agent, from those only 40% were showed growth. Then growth colonies identified by Gram's stain, methylene blue and sub cultured in serum for germ tube test and on chromogenic *Candida* agar media .There gave light green colonies were *Candida albicans* with (28%), which were the most prevalence strain of isolated *Candida* species, followed by *Candida tropicalis* which gave blue colonies with (8%) and then *Candida kruzei* with (4%) *Candida tropicalis* which gave creamy-moove colonies.

Leukemic cancer patient were the most affected group among other patients diagnosed with different cancer types with (13%).

The study showed that cancer patients that resident in Omdurman city and those from Gail tribe were the most affected with an oral candidiasis.

ملخص الاطروحة

في هذه الدراسة تم جمع مائة عينة من مسحة الفم من مرضى السرطان السودانيين الجنسية الذين تم تشخيصهم بمختلف انواع السرطانات والخاضعين للعلاج الكيمائي او الاشعاع او الأثنين معا المتواجدين في مستشفى الخرطوم لعلاج الاورام المعروف سابقا بمركز الخرطوم للعلاج بالأشعة والطب النووي(مستشفى الذرة). لقد تم اخذ العينات من مختلف الفئات العمرية التي تراوحت مابين(2-83) عام ,من كلا الجنسين (66 ذكر و34 انثى) بهدف الكشف عن وجود الأبيضاض الفموي الذي تحدثه فطريات الكانديدا وذلك في الفترة من شهر مايو وحتى يوليو 2015.

بينت الدراسة ان 40% من المرضى اصيبوا بالابيضاض الفموي الفطري . واظهرت الدراسة ان الفئة العمرية التي تحتوي على مرضى اعمارهم اقل من 20 سنة هم الاكثر اصابة بالابيضاض الفموي وان الذكور هم الاكثر اصابة من الاناث وذلك بنسبة 23% .

تم تزريع جميع العينات في السابرود ديكستروز مضافا اليها الكلوروم فينيكول كمضاد بكتيري ووجد فقط ان 40% من التزريع كان ايجابي ثم بعد ذلك تم التعرف على الفطر بعمل صبغة جرام ، والمثلين الأزرق ثم التزريع في السيروم لاختبار تكون النتوء الانبوبي ومن ثم التزريع في الكروموجين اجار المخصصة للكانديدا حيث انها تعطي الوان مختلفة تدل على نوع الكانديدا فوجد ان المستعمرات الخضراء اللون هي الكانديدا البيكان بنسبة 28% وهو اكثر الانواع انتشارا يليها الكانديدا المدارية زرقاء اللون بنسبة 8% ثم الكانديدا كروزي البيجية المائلة الى اللون البنفسجي بنسبة 4%.

اظهرت الدراسة ان مرضى الليوكيميا هم الأكثر اصابة بالأبيضاض الفموي من المرضى المصابين بانواع السرطانات الأخرى.

اظهرت الدراسة ان المرضى المقيمين في مدينة امدرمان هم الأكثر اصابة بالابيضاض الفموي الفطري وأن المرضى المنحدرين من قبيلة الجعل هم الأكثر اصابة بالابيضاض الفموي.

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List of abbreviations

HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immune Virus
ALL	Acute Lymphoid Leukemia
AML	Acute Myeloid Leukemia
CML	Chronic Myeloid Leukemia
CLL	Chronic Lymphoid Leukemia
HL	Hodgkin's Lymphoma
NHL	None- Hodgkin's Lymphoma
LBCL	Large B cell Lymphoma
CRC	Colorectal Cancer
VVC	Volvo Vaginal Candidasis
TNM	Tumor-node-metastasis
CT	Computed Tomography
MRI	Magnetic resonance Image
PAP	Papanicolaou
HPV	Human Papilloma Virus

Chapter One

Introduction

1. Introduction

1.1 Oral candidiasis

Is the most prevalent opportunistic infection affecting oral mucosa. In the vast majority of cases, the lesions are caused by the yeast *Candida albicans* .The pathogenesis is not fully understood, but a number of predisposing factors have the capacity to convert *Candida* from the normal commensal flora (saprophytic stage) to a pathogenic organism (parasitic stage).*Candida albicans* is usually a weak pathogen affect the very young, the very old and the very sick. Most *Candida* infections only affect mucosal linings but the rare systemic manifestation may have a fatal course. Oral Candidiasis is divided in to primary and secondary infection .The primary infections are restricted to the oral and the perioral sites, but secondary infections are a accompanied by systemic mucocutaneous manifestation (Martin *et al*., 2008).

Genus *Candida* is composed of an extremely heterogeneous group of organism which grow as yeast (blastoconidia).Most members of the genus produce filamentous type of growth as pseudohyphae or even true hyphae. Some species have ascomycetous sexual stage. *Candida* is symbiotic yeast that is found in the environment as soil, water and plants. *Candida* is a component of normal flora of human and animals. Many of them cause Candidiasis as superficial or invasive forms. Invasive form is common in immunocompromized or debilitated individuals (Taha, 2011).

1.2 Rationale

Treatment of cancer is increasingly effective but is associated with short and long term side effects. Oral side effects, including oral Candidiasis, remain a major source of illness despite the use of variety of agents to treat them .Change in the host immunologic defence mechanisms which compromised by cancer and cancer treatment are contribute in occurrence of an over growth of *Candida* species, this might spread from the mouth to pharynx and oesophagus, gastro intestinal tract and blood stream causing severe symptoms such as erosion and ulcerations of the tissues. Complication of an oral Candidiasis when it reaches blood and lung can lead to death.

1.3 Objectives

1.3.1 General objectives

To determine possible relation between cancer patients who were received treatment and oral Candidiasis.

1.3.2 Specific objectives

To estimate the percentage of oral Candidiasis in cancer patients

To estimate the most prevalent strain of *Candida* species among cancer patients

To evaluate that which type of cancer patients susceptible to an oral Candidiasis

To detect relation between oral Candidiasis in cancer patients and risk factors (Locality, Tribe and Gender).

Chapter Two

Literature Review

Chapter Two

2. Literature review

2.2 Predisposing factors lead to oral Candidiasis

This divided in to local and general factors

The presence of commensal bacteria in the mouth makes it difficult for invading microorganisms to become established, but change in oral flora upset this balance. For instance:-

2.1.2 General factors

Often related to the patient's immune system and endocrine status, the immune status can be affected by drugs as well as disease, which suppresses the adaptive or innate immune system this include:-

-Prolonged administration of broad spectrum antibiotics allows the normally harmless *C. albicans* to flourish penetrating the epithelium with the pseudomycelia.

-Impaired immunity, as in HIV infection, malignancy, and occasionally in new born infants and the elderly.

-Chemo therapy.

-Immune suppressive drugs.

-Endocrine disorder.

-Hematinic deficiencies. (Dockrell *et al*., 2004).

2.1.2 Local predisposing factors

Are able to promote growth of *Candida* or to affect the immune response of the oral mucosa .This include:

-Denture wearing

-Smoking

-Atopic constitution

-Inhalation steroids

-Topical steroids

-Hyper keratosis

-Imbalance of oral micro flora

-Quality and quantity of saliva (Burket *et al*. 2008)**.**

2.2 *Candida* species

Genus *Candida* comprises about 170 species (formerly 200 or 250 *Candida* species was reported, but recently, according to molecular biology, the number of species is restricted).The most medically important *Candida* species are:

2.2.1 *Candida albicans*

Candida albicans is the most important cause of superficial forms of Candidiasis mostly the individual's own endogenous reservoir as mouth, gastro intestinal tract, lower genital tract and skin.

2.2.1.1 Macromorphology

The colonies of *Candida albicans* are creamy colour, pasty and smooth, grow in 48-72 hours on Sabouraud's dextrose agar at 25 c and 37 c. On chromogenic *Candida* agar media, it produces light green or green colonies.

2.2.1.2 Micromorphology

On corn meal or rice agar it produce true hyphae, pseudohyphae, clusters of blastoconidia and chlamydoconidia. A variant of *Candida albicans,* previously known as *Candida* African, does not produce chlamydospores.

Germ tube formation: *Candida albicans* incubated for 2 hours in 10% serum at 37 c produce typical cell elongation (germ tube) with the absence of constriction at the tube base.

2.2.1.3 Physiological and biochemical characters

Candida albicans have the ability to grow at 42 c and in the presence of cyclohexamide.It ferments glucose, galactose and maltose while assimilating glucose, galactose, sucrose and maltose. (Taha, 2011).

2.2.2 *Candia dubliniensis*

Candida dubliniensis is closely related to *Candida albicans* since the yeast grow as pasty creamy colonies on sabouraud's dextrose agar (SDA),on corn meal agar pseudohyphae and clusters of chlamydospores are formed and in 10% serum at 37 c germ tube are produce. The difference between the two species is in colour of colony on chromogenic agar media which is Dark green while in *Candida albicans* is light green and it cannot assimilate xylose and glycosidase as *Candida albicans do*. It cannot grow at 42 c while *Candida albicans do.* It also can assimilate glucose, galactose, sucrose and maltose like *Candida albicans.(Taha,2011)*

2.2.3 Candida tropicalis

2.2.3.1 Macromorphology

On Sabourauds's dextrose agar colonies are creamy to white,glistening or dull, soft and smooth. On chromogenic *Candida* agar, colonies appear as dark blue to blue grey.

2.2.3.2 Micromorphology

Candida tropicalis appears as ovoid to elongate yeast cells when cultured on Sabouraud's dextrose agar. On corn meal medium, it produces long branched pseudohyphae with small blastoconidia which occurs singly or in clusters. True hyphae also may be occur.C.tropicalis does not produce chlamydoconidia although round to pear shaped cells may be present at the tops of pseudohyphae which have thin wall.

2.2.3.3 Physiological and biochemical characters

The yeast at 37 C but not at 42 C and can grow on the presence of cyclohexamide.It does not hydrolyze urea. It ferments glucose, galactose ,maltose ,trehalose and sometimes sucrose while assimilating glucose ,galactose ,sucrose and maltose. Sucrose negative variants of *Candida tropicalis* have been found known as *C.paratropicalis.* (Taha, 2011).

2.2.4. *Candida parapsilosis*

2.2.4.1 Macromorphology

On Sabouraud's dextrose agar,colonies are white to creamy smooth shiny to dull and may be wrinkled .On chromogenic *Candida* agar, appears as white colonies to pale pink

2.2.4.2 Micromorphology

C.parapisilosis appears as oval elliptical and elongated cells in a film made from colonies on Sabouraud's dextrose agar. On corn meal agar, pseudohyphae consist of regular branches (pine forest) with chains of elongated or clusters of blastoconidia are found. The pseudohyphae may be curved and large hyphal cells called giant cells could be observed.

2.2.4.3 Physiological and biochemical characters

Candida parapsilosis grow at 37 C but not at 42 C .It cannot grow in the presence of cyclohexamide ,does not hydrolyse urea, ferments glucose and may be galactose while assimilating glucose, galactose, maltose and trehalose.(Taha,2011).

2.2.5 Candida krusei

2.2.5.1 Macromorphology

Colonies on Sabouraud'S dextrose agar are flat, while dull or even buff with smooth margin. On chromogenic *Candida* agar, colonies appears as pale pink or purple with rough surface.

2.2.5.2 Micromorphology

The yeast appears as oval to elongate cells. On corn meal ,pseudohyphae with elongated blastoconidia are found in clusters .The pseudohyphae are mostly primitive and some strains fail to produce it.

2.2.5.3 Physiological and biochemical characters

Candida krusei grow at 37 c but not at 42 c .It cannot grow in the presence of cyclohexamide. Some isolate hydrolyse urea. It ferment glucose but fail to ferment other sugars .It assimilates glucose and fail to assimilate other sugars.(Taha,2011).

2.2.6 *Candida glabrata*

2.2.6.1 Macromorphology

On Sabouraud's dextrose agar (SDA) it is white to creamy colour with small size usually smooth, soft and glistening. On chromogenic *Candida* agar colonies appear as pink colour.

2.2.6.2 Micromorphology

It appears as ovoid cells with small size .On corn meal agar, no pseudohyphae or chlamydospores produced.

2.2.6.3 Physiological and biochemical characters

The yeast grows at 37 c but not at 42 c and cannot grow in the presence of cyclohexamide. Urease test is negative ,it ferments glucose only and assimilate glucose and trehalose.(Taha,2011).

2.2.7 *Candida Lusitania*

2.2.7.1 Macromorphology

Colonies on (SDA) at 25 c are white to creamy colour, soft, smooth and shiny. On chromogenic *Candida* agar, pink or purplish greyish colonies appeared.

2.2.7.2 Micromorphology

On corn meal agar short highly branched (bushy) pseudohyphae with abundant blastoconidia along the length of the hyphae.

2.2.7.3 Physiological and biochemical characters

It grows at 37 c but not at 42 c .It does not grow in the presence of cyclohexamide, does not hydrolyse urea, ferments glucose and galactose while assimulating glucose, galactose, maltose and trehalose.Since it is similar to *C.parapsilosis*,*Candidalusitania* is differentiated from *C.parapsilosis* by its ability to both ferment and assimilate cellulose as well as assimilation of rhaminose.(Taha,2011).

2.2.8 *Candida guilliermondii*

2.2.8.1 Macromorphology

On (SDA), it is dull, white, creamy or yellow, flat, smooth colonies .On chromogenic *Candida* agar, pale pink to purple colonies appeared.

2.2.8.2 Micromorphology

On corn meal agar, short branched pseudohyphae is seen, bearing clusters of blastoconidia.

2.2.8.3 Physiological and biochemical characters

It grows at 37 c but not at 42 c, does not hydrolyse urea. It ferments glucose and fail to ferment galactose, sucrose, maltose and lactose while assimilating glucose, galactose, sucrose and maltose. (Taha, 2011).

2.2.9 *Candida kefyrl*

2.2.9.1 Macromorphology

On (SDA), colonies are creamy colour and smooth. On chromogenic *Candida* agar, pink to purple colonies are produced.

2.2.9.2 Micromorphology

It shows oval to elongated yeast cells. On corn meal agar ,elongated blastoconidia at the constriction of pseudohyphae are noticed, then they separate and lie parallel to it ,so called log jam.

2.2.9.3 Physiological and biochemical characters

It grows in the presence of cyclohexamide. Its growth at 37 c is variable .Urea is not hydrolysed .It ferment glucose, galactose and with variable results with sucrose, maltose and lactose, It assimilates glucose, galactose ,sucrose ,lactose and raffinose and with variable results with trehalose (Taha,2011).

2.3 Classification of *Candida*

Yeasts are traditionally classified in to ascomycetous,basidiomycetous and deutromycetous according to their sexual reproduction .Recntly it was found that yeasts do not have perfect state (telophase) found to be related phylogenetically to ascomycetes or basidiomycetes.so,yeasts are classified now in to tow groups only ascomycetous and basidiomycetous.*Candida* yeast that of interest of this research is Ascomycetous yeast.(Taha,2011)

2.3.1 Ascomycetous yeast:

It is found in two classes of Ascomcota namelyEndomycetes and pneumocyst-idiomycetes.Ascomycetous yeast characterized by noncapsulated, fermentative, nonin-ositol assimilative, negative urease, not produce pigment and negative for diazonium blue bsalt (DBB).The medical important ascomycetous yeast genera are:

-*Candida*

-*Gearichum*

-*blastochizomycetes*

-Pneumocystes. (Taha, 2011)

2.3.2 Class Endomycetes has two orders:

Saccharomycetales and endomycetales

2.3.2.1. Order Saccharomycetales

- Family Saccharomycetaceae: Characterized in it anamorphic with budding cells.it must be mentioned that non sexual form of Saccharomyces and some *Candida* species which found in genera Deparomyces ,pichia,Klaryvermyces. It must be mentioned that non sexual *Candida* species are also related phlogenitically to saccaromycetaceae.
- familyDipdoascaceae:Characterized by arthroconidia and contain genus Blastoschizmycetes .(Taha,2011).

2.3.2.2 Order Endomycetales

Contain family endomycetaceae in which genus Galaclomyces,the perfect state of Geotrichum is classified

2.3.3Class Pneumocystidiomycetes

Contain order pneumocystidales which have one family pneumocystideaceae in which found genus pneummocystis (Taha, 2011).

2.4 Cancer

Is an abnormal growth of cells caused by multiple changes in gene expression leading to disregulated balance of cell proliferation and cell death and ultimately evolving in to a population of cells that can invade tissues and metastasize to distant site ,causing significant morbidity and if not treated death of the host .Cancer is a disease of a higher multi cellular organisms .The characteristics that delineate a malignant cancer from a benign tumour are the abilities to invade locally ,to spread to regional lymph nodes and metastasize to distant organs in the body ,Clinically cancer appear to be many different disease with different phenotyping characteristics .As a cancerous growth progresses ,genetic drift in the cell population produces cell heterogeneity in cell characteristic as cell antigenicity, invasiveness ,metastatic potential ,rate of cell proliferation ,differe-ntiation state ,and response to chemotherapeutic agents, At the molecular level ,all cancers have several things in common ,which suggest that the ultimate biochemical lesions leading to malignant transformation and progression can be produce by a common but not identical pattern of alteration of gene readout .In general malignant cancer cause significant morbidity and will be lethal to the host if not treated .exception to this appear to be latent ,indolent cancer may remain clinically undetectable, allowing the host to have standard life expectancy.(Raymond,2007).

2.4.1 Types of malignant cancer

2.4.1.2 Hematological malignancies

2.4.1.2.1 Leukemia's

They are disease with an incidence of about 10 per 100 000 per yea .They are classified as being acute(short natural history) or chronic (long natural history),and of myeloid or lymphoid origin More than half of the leukemia's present acutely (Acute lymphoid leukemia and Acute myeloid leukemia) with the remainder being chronic types(Chronic lymphoid leukemia and Chronic myeloid leukemia).The type of leukemia's varies with age ,acute lymphoblastic leukemia (ALL) is mainly seen in childhood and chronic lymphocytic leukemia is a disease of the elderly .Leukemia can be

diagnosed by examination of stained slide of peripheral blood and bone marrow ,with immune phenotyping ,cytogenetic and molecular genetics being essential complete sub classification and prognostication.(Kumar and Clark,2005).

2.4.1.2.1.1 General classification

The characteristics of leukemic cells can be assessed by light microscopy, expression of cytosolic enzymes and expression of surface antigens .These will reflect the lineage and degree of maturity of the leukemic clone .Thus, and leukemia can be divided on the basis of the speed of evolution of the disease in to acute or chronic. Each of these is then further subdivided into myeloid or lymphoid according to the cell type involved.

-Acute myeloid leukaemia (AML)

-Acute lymphoblastic leukaemia (ALL)

-Chronic myeloid leukaemia (CML)

-Chronic lymphocytic leukaemia (CLL). (Kumar and Clark, 2005).

2.4.1.2.2 Lymphomas

These are more common than leukeamias and are increasing in incidence for reasons which are unclear. They arise as the result of abnormal proliferation of the lymphoid system, and hence occur at any site where lymphoid tissue is found. Most commonly they are manifest by the development of lymphadenopathy at single or multiple sites, although primary extranodal presentations account for up to 20% of non-Hodgkin's lymphoma .The prognosis is determined by the specific subtype of lymphoma and the anatomical extent of disease and its bulk, the clinical course ranging from month to years . (Kumar and Clark, 2005).

Lymphomas are currently classified on the basis of histological appearance into:

2.4.1.2.2.1 Hodgkin's lymphoma (HL)

This is a rare disease involving primarily the lymph nodes.

Aetiology

There is epidemiological evidence linking previous infective mononucleosis with HL and up to 40% of patients with HL have increased EBV antibody titers at the time of diagnosis and several years prior to the clinical development of HL. These data suggest a role for EBV in pathogenesis. Other viruses have not been detected .Other environmental and occupational exposure to pathogens has been postulated. (Kumar and Clark , 2005).

2.4.1.2.2.2 Non-Hodgkin's lymphoma (NHL)

These are malignant tumors of lymphoid system classified separately from Hodgkin's lymphoma. Most (70%) are of B cell origin although T cell tumors are increasingly being recognized. There is slight male predominance. The median age of presentation is 55-75 years.

Aetiology

The cause is unknown .There is wide geographical variation which probably reflects different environmental factors .NHL is associated with the EBV virus (Burkitt's lymphoma) and the T cell lymphocytic virus which is prevalent in Japan ,Africa, South America and Caribbean .Herpes virus 8 is associated with primary effusion lymphomas and castleman's disease, there is an increase in lymphoma in patients with AIDS .Helicobacter pylori is an aetiological factor in gastric MALT lymphoma .Lymphomas also occur in congenital immunodeficiency, post-transplantation and in autosomal family cancer syndrome. Other causes, e.g. occupation, dietary and exposure to chemicals, have been linked to the increasing incidence but the evidence is unconfirmed.

Pathogenesis and cytogenetic features

Translocation of chromosomes result in the presence of different type of lymphomas. There is a malignant clonal expansion of lymphocytes which might occur

at different stage of lymphocyte development .in general ,neoplasm of non dividing mature lymphocytes are indolent whereas those proliferating cells 9 eig. Lymphoblast , immunoblast) are much more aggressive .This malignant transformation is usually due to errors in gene rearrangements which occur during the class switch, or gene recombinations for immunoglobulins and T cell receptors. Thus, many of the errors occur within immunoglobulin loci or T cell receptor loci. For example, an abnormal gene translocation may lead to the activation of a proto-oncogene next to a promoter sequence for the immunoglobulin heavy chains (ig-H).

Burkitt's lymphoma was the first tumour in which a cytogenetic change was shown to involve the translocation of specific gene. The most frequent change is a translocation between chromosome 8 and 14 in which the myc oncogene move from chromosome 8 to a position near the constant region of immunoglobulin heavy chain gene on chromosome 14,resulting in up regulation of myc. Similar rearrangements involving the light chain loci are seen in the alternative Burkitt's lymphoma translocation between chromosome 8 and either chromosome 20e 22.other somatic cytogenetic abnormalities associated with human lymphoma are the t(14,18) in follicular lymphoma, involving up regulation of bcl-2 gene or up regulation of Bcl-1 also called cyclin D10 as a result of t(11,14)in mantle cell lymphoma.(Kumar and Clark,2005).

2.4.1.2.2.2.1 Types of non Hodgkin's lymphoma

2.4.1.2.2.2.1.1 Follicular lymphoma

This comprise20% of all B cell lymphoma . Most patients with follicular lymphoma present feeling well but with painless lymphadenopathy .Investigation usually reveals multiple sites of disease: involvement of bone marrow is common. Managed conservatively it is a remitting and recurring disease with a clinical course running over a median of 10(1-20 years) years during which there will be about three episodes of relapse .Death occur because of resistant disease, transformation to diffuse large B cell lymphoma (LBCL) or the effects of therapy .The well patient should be managed with no specific therapy until progression is documented. Repeat biopsy should be performed at

this time in case there has been histological transformation to LBCL as this has specific implication for therapy. (Kumar and Clark , 2005).

2.4.1.2.2.2.1.2 Lymphoplasmacytic lymphoma

This is an uncommon B cell lymphoma often presenting with heavy bone marrow infiltration, and is almost the only lymphoma to be diagnosed on bone marrow biopsy alone. There is frequently splenomegaly and anemia, and in some an associated paraproteinig m with associated immune paresis (Waldenstrom's macroglobulinaemia , WM) occurs .Patients in this group are usually older and commonly present with the symptoms of bone marrow failure or hyperviscosity.it may be a chance diagnosis. Management may be expectant, the indication for treatment being:

-Symptomatic anemia

-Recurrent infection

-Symptoms of hyperviscosity, e.g. headache, visual disturbance

-Progression. (Kumar and Clark , 2005).

2.4.1.2.2.2.1.3 Mantle cell lymphoma

This is an uncommon lymphoma (6% of non –Hodgkin's lymphoma) with a median survival of 3-4 years .It occurs predominantly in the elderly and almost always widely disseminated at presentation, with lymphadenopathy and frequent involvement of the bone marrow and gastrointestinal tract. Treatment is often compromised by co-morbidity. (Kumar and Clark , 2005).

2.4.1.2.2.2.1.4 Large B cell lymphoma (DLBCL)

This is the commonest lymphoma and is almost invariably fatal without therapy within months, and was previously classified as aggressive or high grade lymphoma. Now more than 50% of young patients are cured. The only indications for palliative

approach at the initial presentation are extreme co-morbidity and will of the patient. Expectant management is inappropriate. Patients present with rapidly progressive lymphadenopathy and progressive infiltration of many organ, e.g. spinal cord, gastrointestinal tract. (Kumar and Clark, 2005) .

2.4.1.2.2.2.1.5 Burkitt's lymphoma

This is an uncommon lymphoma in western world>It is endemic to Africa in the mosquito belt: there is a close association with the Epstein-Barr virus and it is a disease with very high proliferative index which is very rapidly fatal without therapy. The clinical presentation worldwide is usually that of lymphadenopathy often with abnormal mass and frequently with bone marrow infiltration (and leukemia).Central nervous system involvement is common, up to 30% having meningitis at the time of presentation. In Africa, by far the two commonest presentations are the abdominal mass or a large tumor involving the jaw< the bone marrow and central nervous system are also frequently involved. (Kumar and Clark , 2005).

2.4.1.2.2.2 1.6 T cell lymphomas

These are much less common than |than the B cell counterparts. In the main ,the overall treatment strategies are the same, but success is much more limited. (Kumar and Clark, 2005) .

2.4.1.2.2.2.1.7 Primary extranodal lymphoma

The WHO classification does not distinguish between primarily nodal or extranodal at the time of presentation if the histological pictures is the same .Which classified in to :

-Primary cerebral lymphoma

-Primary gastric lymphoma

-Primary cutaneous lymphoma. (Kumar and Clark, 2005) .

2.4.1.2.2.2.1.8 Multiple myeloma

Myeloma is a malignant disease of the plasma cells of bone marrow, accounting for 1% of all malignant disease. There is a clonal expansion of abnormal, proliferating plasma cells producing a monoclonal paraportion, mainly Ig G (55%) or Ig A (20%) and rarely Ig D .The paraproteinaemia may be associated with excretion of light chains in the urine (Bence Jones protein), which are either kappa or lambda. In approximately 20% there is no paraproteinaemia, only light chains in the urine. (Kumar and Clark , 2005).

2.4.1.3 Solid tumors

This includes the following types:

2.4.1.3.1 Lung cancer

This divided into two types

2.4.2.1.2 Non-small-cell lung cancer (NSCLC)

The staging is classified according to the TNM system by which the disease can be divided into local, locally advanced, and advanced stages with 5-years survival varying from 55-67%, to 23-40%, to 1-3% respectively. The addition of CT and PET scanning has increased the accuracy of staging and improved the selection of patients for surgery and adjuvant therapy. (Kumar and Clark, 2005).

2.4.2.1.3 Small-cell lung cancers (SCLC)

The staging of small-cell lung cancer is divided into limited and extensive disease according to whether or not it is confined to single anatomical area or radiation field. (Kumar and Clark, 2005) .

2.4.2.2 Breast cancer

Breast cancer is the most common cancer in women who do not smoke .The screening programme in the UK, with mammography every 3 years in women aged 50-64 and improvements in multimodality treatment have improved overall survival and rates

of cure, while breast-conserving surgery has greatly ameliorated the psychosexual impact of the disease .The size of the primary tumour, the histological subtype (most are infiltrating ductal carcinoma), histological grade, differentiation, oestrogen and progesterone receptor status, patients age and menopausal status are all significant independent predictors of risk of recurrence. Expression of c-erbB2 is linked to the above and predictor of treatment response. (Kumar and Clark , 2005).

2.4.2.3 Gastrointistinal cancer

This include:

2.4.2.3.1 Oesophageal cancer

Histological ,stage ,age and performance status are critical to treatment decisions, which should be made by a multidisciplinary team in designated units .The prognosis for the majority of symptomatic patients is poor,50% have distant metastases at the time of diagnosis and the majority of the remainder will have loco-reional spread into mediastinal structure. (Kumar and Clark , 2005).

2.4.2.3.2 Gastric cancer

Neoadjuvant therapy for potentially resectable squamous carcinomas with cisplatin, 5-flurouracil and concurrent radiotherapy achieves complete remission in 20-40% with 25-35% of patients alive 5-years after surgery. (Kumar and Clark, 2005) .

There is increased perioperative mortality. Postoperative chemotherapy for adenocarcinoma (Kumar and Clark, 2005).

2.4.2.3.3 Colorectal cancer

Colorectal cancer (CRC) is the most common cause of cancer death in the UK. Each year over 30 000 new cases are diagnosed in England and Wales (68% colon, 32% rectal cancer) and it is registered as the underlying cause of death in about half this number. The prevalence rate per 100 000 (all ages) is 53.5 for men and 36.7 for women .The incidence increases with age, the average age at diagnosis being 60-65 years .The

disease is much more common in westernized countries than in Asia or Africa. Site of the disease, above or below the pelvic peritoneal reflection, and TNM stage are the main prognostic factors. (Kumar and Clark, 2005).

2.4.2.3.4 Epithelial ovarian cancer

Epithelial ovarian cancer comprise 80% of all ovarian cancers, the remainder being of germ cell or stromal origin. (Kumar and Clark , 2005).

2.4.2.3.5 Prostate cancer

It account for 7% of all cancers in men and is the fourth most common cause of death from malignant disease in men in England and Wales. Malignant change with advancing age, by the age of 80 years, 80% of men have malignant foci within the gland ,but most of these appear to lie dormant histologically. The tumour is an adenocarcinoma. Hormonal factors are thought to play a role in the aetiology. It divides in to early prostate cancer and advanced prostate cancer. (Kumar and Clark, 2005).

2.4.2.3.6 Testicular and ovarian germ cell tumours

Germ cell tumours are the most common cancers in men aged 15-35 years but comprise only 1-2% of all cancers .They are much less common in women .there are two main histological types, seminoma (dysgerminoma in women) and teratoma. Teratomas may comprise varying proportions of mature and immature elements. Germ cell tumors may rarely occur in extragonadal sites in the midline from pituitary, mediastinum or retroperitoneum but should be treated in a similar manner. (Kumar and Clark , 2005).

2.4.2.4 Liver tumours

The most common liver tumour is a secondary (metastatic) tumour, particularly from the gastrointestinal tract, breast or bronchus. Clinical features are variable but usually include hepatomegaly. Ultrasound is the primary investigation, with CT or MRI used when available , MRI is comparable to CT at detecting metastases. Primary liver tumours may be benign or malignant, but the most common are malignant. (Kumar and Clark , 2005).

2.4.2.4.1 Malignant liver tumour

2.4.2.4.1.1 Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) is one of the 10 most common cancers worldwide .Carries of HBV and HCV have an extremely high risk of developing HCC. In areas where HBV is prevalent,90% of patients with this cancer are positive for hepatitis B virus. Cirrhosis is present in over 80% of these patients .The development of HCC is related to the integration of viral HBV DNA in to the genome of the host hepatocyte and possibly the degree of viral replication .The risk of HCC in HCV is a high as or higher than in HBV despite no viral integration. Primary liver cancer is also associated with other form of cirrhosis, such as alcoholic cirrhosis and haemochromatosis. Males are affected more than females , this may account for high incidence seen in heamochromatosis and low incidence in PBC. Other suggested aetiological factors are aflatoxin (a metabolite of a fungus found in groundnuts) and androgenic steroids, and there is a weak association with the contraceptive pill.Thetumour is either single or multiple nodules throughout the liver. Histologicaly it consists of cells resembling hepatocytes. It can metastasize via the hepatic or portal veins to the lymph nodes, bones and lung. (Kumar and Clark, 2005) .

2.4.2.4.1.2 Hydatid disease

Cyst caused by Echinococcusgranulosus are single or multiple. They usually occour in the lower part of the right lower lobe of the liver .The cyst has three layers: an outside layer derived from the host, an intermediate laminated layer and an inner germinal layer that buds off broad capsules to form daughter cysts. Clinically there may be no symptoms or a dull ache and swelling in the right hypochondrium .Investigations show a peripheral eosinophilia in 30% of cases and usually a positive hydatid complement-fixation test or haemagglutination (85%).Plain abnormal x-ray may show calcification of the outer coat of the cyst .Ultrasound and CT scan demonstrate cyst and may show diagnostic daughter cysts within the parent cyst .Medical treatment (e.g. with albendazole 10 mg/kg ,which penetrate in to large cysts) can result in reduction of cyst size. (Kumar and Clark, 2005) .

2.4.2.5 Cervix cancer

Cervical cancer is the second-most common cancer in the world .More than 500,000 women worldwide are diagnosed each year with this disease and nearly half of those will die from it .Before the invention of the PAP smear procedure in 1940s, cervical cancer was the most deadly form of cancer for women in the United States .Fortunately ,cervical cancer usually develop slowly ,over the course of months or even years so with regular screening it is possible to detect this cancer in the earliest stages when it is most easily treated .Before cancer appear in cervix, there are typically precancerious changes ,known as dysplasia ,which may occur .Cervix cancer often has no symptoms so it is more important for women to have regular gynaecological examination (Spencer,2009).

2.4.2.6 Osteosarcoma

Although it relatively a rare neoplasm. It does represent the most common bone malignancy in childhood with an incidence of 4-5 per million populations. Diagnosis and classification of osteosarcoma relies on correlation of biopsy finding with imaging studies, while molecular finding continue to be of limited value in the diagnosis of osteosarcoma (Heymann, 2009).

2.4.2.7 Papillary Thyroid Cancer

The natural history and prognosis of full differentiated thyroid cancer has been intensively studied since the 1980s .A clear differentiation of risk factors associated with poor outcome have allowed more selective and less aggressive treatment recommendation. In general well differentiated thyroid cancer is one of the least morbid solid carcinomas, with favorable long term survival. However small proportion of patients with papillary cancer and slightly larger proportion of patients with follicular thyroid cancer die from disease related cancers.(DeVita.,*et al*.2008)

2.4.2.8 Rhabdomyosarcoma (RMS)

Rhabdomyosarcoma is an embryonal sarcoma derived from primitive mesenchymal cells throughout the body that shows evidence for muscle differentiation

within the mass .It is the most common soft tissue sarcoma in children.Two histological types are seen embryonal and alveolar rhabdomyosarcoma.Onlyembryonal RMS has been reported in the central nervous system.RMS is a childhood tumor with a median age of 10-10.5 years old (Kesari., *et al 2008).*

2.4.2.9 Pyriform fossa

Pyriform fossa cancers represent approximately 7% of all cancers of the upper acro-digestive tract and 70% of hypopharyngealcancers.Men are much more affected than women and are typically 55-70 years of age at presentation .Risk factors for the development pyriform fossa squamous cell carcinoma as with larynx and other sites of the pharynx include tobacco smoking , alcoholingestion ,diet lacking in fresh fruit and vegetables , gastroeseophageal reflux disease and HPV 16 seropositivity .It is interesting to note that people in a good Mediterranean diet have less than half the risk of developing pharyngeal cancers and that a high intake of red meat ,processed meat and fried foods increase the risk of pharyngeal cancer (Gullane .,*et al*2012).

2.5 Cancer Treatments

2.5.1 Chemotherapy

Is the treatment of malignant disease with antiproliferative agents. Most of these drugs cause non- specific DNA damage ,either leading to cell death by apoptosis or preventing cell division .The majority of cell biochemical process are identical in normal and malignant cells, but malignant cell are characterized by uncontrolled proliferation and may fail to recognize and repair DNA damage .In contrast, normal tissues exposes to chemotherapy demonstrate temporary loss of proliferating cells ,with a variety of side-effects but then recover through damage repair or replacement of cells from normal precursor or stem cells. (Wilkins, 2011).

2.5.1.1 Classes of chemotherapy agents by mechanisms of action

DNA binding:- direct alteration of DNA by alkylating agent (e.g.nitrogenmustard, cyclophosphamide)

Antimetabolites:- block synthesis of purines and pyrimidines ,essential for DNA synthesis (e.g.5-flurouracil,methotrexate)

Antimicrotubule:-interfere with mitosis (e.g. vincaalkaloids , taxanes)

Topoisomerase: - inhibition lead to DNA damage. (Wilkins , 2011).

2.5.1.2 Chemotherapy side-effects

Many such drugs affect organs which are dependent bin cell renewal to maintain tissue integrity:

Bone marrow: neutropenia , infection ,thrombocytopenia ,anaemia

Gastrointestinal tract : nausea ,vomiting ,diarrhoea ,mouth ulcer

Skin: hair loss

Gonads: infertility

Others have more specific normal tissue effects:-

Antimicrotubule: peripheral nerve damage

Anthracycline: cardiomyopathy. (Wilkins, 2011).

2.5.1.3 Chemotherapy dose and scheduling

Generally, increasing doses result in increasing cell kill both normal and tumour tissues .Chemotherapy is commonly delivered at the highest safe dose once every 2-4 weeks ,in order to allow normal tissue recovery, in particular bone marrow .however some drugs (e.g.5-flurouracil)are administered continuously at low dose with relatively reduced side-effects but increased tumour cell kill.(Wilkins,2011).

2.5.2 Radiotherapy

Is a treatment involving the use of high energy radiation, this damage the DNA of cancer cells causing them death ,nearby healthy tissues also suffer temporary cells damage from radiation but these cells are usually able to repair their damage DNA and continue to proliferate normally, it is commonly used to treat cancer ,almost half of all

people with cancer have Radiotherapy as part of their treatment plan .Radiotherapy is sometimes used to treat benign (non-cancerous) tumour and other condition, such as thyroid disease and other blood disorders. radiotherapy can be used alone or in combination with chemotherapy to try cure cancer, for people with incurable cancers, radiotherapy is a very effective way of controlling symptoms .radiotherapy can also use before surgery to shrink tumour so it is easier to remove (known as neoadjuvant treatment),or after surgery to remove small amounts of tumour that may be left .(Wilkins,2011).

2.6.2.1 Types of radiotherapy

2.6.2.1.1 External radiotherapy given from outside the body, it is usually involve using a machine called a linear accelerator which focuses high energy radiation beams on to the area requiring treatment. External beam radiotherapy is completely painless and it is usually involves a series of daily treatments over a number of days or weeks. (Wilkins , 2011) .

2.6.2.1.2 Internal radiotherapy given from inside the body, it involveplacing a small piece of radioactive material temporarily inside the body near the cancerous cells (known as brachy therapy), or the use of a radioactive liquid that's swallowed or injected. The radiation emitted by internal radiotherapy is painless, though the procedure to insert the source can sometimes cause mild discomfort. The type of radiotherapy you have and the length of treatment depends on the size and type of cancer, and where it is in your body. (Wilkins , 2011) .

2.6.2.1.3 Side effects of radiotherapy

The temporary DNA damage to normal tissues causes side effects, most of which are short-lived. Common side effects include:-These tend to get better within a few days or weeks of treatment finishing.

-Sore skin

- Tiredness

-Hair loss

-Permanent infertility. In rare cases . (Wilkins , 2011).

2.6.3. Sabouraud's dextrose agar:

Sabouraud (pronounced sah-bū-rō′) agar medium was developed by the French dermatologist Raymond J. A. Sabouraudin the late 1800'sto support the growth of fungi that cause infection of the skin, hair, or nails, collectively referred to as dermatophytes Sabouraud's medical investigations focused on bacteria and fungi that cause skin lesions, and he developed many agars and techniques to culture pathogenic moulds and yeasts, such as dermatophytes and *Malassezia*. He particularly desired that all mycologists detail their exact media formulations, temperatures and times of incubation of specimens, in order to standardize the field's observations and thus reduce differences in appearance as a possible source of error in identification. The medium is complex but contains few ingredients. Peptones, as soluble protein digests, are sources of nitrogenous growth factors that can vary significantly according to protein source. Sabouraud's original formulation contained a peptone termed "Granule de Chassaing," which is no longer available. (This may be why the standard name for this medium is "Sabouraud agar, modified.") Variations in pigmentation and sporulation can be consistently observed if one uses Sabouraud medium prepared with consistent ingredients, because morphology can vary slightly based on the peptones used. Both Difco and BBL Sabouraud agars use pancreatic digests of casein as their peptone source. Although Sabouraud originally used the sugar maltose as an energy source, glucose (or dextrose, as it used to be called), is currently used, and agar serves to solidify the medium (Janelle Hare., 2008).

2.6.4 Germ tube test

2.6.4.1 Purpose

The germ tube test is used in the identification of pathogenic *Candida albicans* species strain and *Candida dubliniensis.*

2.6.4.2 Principle

In the presence of serum *Candida albicans blasto conidia (yeast cells)* produce hyphal elements (germ tubes) more rapidly than do most other species of yeast, unlike some other yeast species, no constriction exist between the *C.albicans* mother cell and the germ tube *(paul G Engelkirket al.,2008).*

2.6.4.3 Reagent

It is best to use fetal or newborn calf serum, but in this study human blood serum were used ,Also in order to release an accurate results examine with positive and negative control organisms.*(* paul G Engelkirk *et al.*,2008*).*

2.6.4.4. Procedure

The procedure is as follow:

1-A small amount of the yeast colony was inoculated in to 0.5 ml of human blood serum.

2- Incubated at 37-36c for up to 3 hours.

3-After incubation, examine a wet preparation of the yeast suspension at ×40 magnification. Look for the characteristic hyphal elements.

2.6.4.5. Interpretation of results

The presence of germ tubes having no constriction at the site of origin is considered a positive test result, consistent with *C.albicans*. However the absence of germ tubes does not rule out *C.albicans .(*paul G Engelkirk*et al.*,2008).

2.6.5 Previous studies

Oral Candidiasis in cancer patients is an infection for which inconsistent diagnostic and therapeutic strategies currently prevail. Recent studies have shown its

potential importance in the development of systemic Candidiasis. A clinical and cytological study was undertaken on 52 consecutive cancer patients admitted to our institution. Although the incidence of clinical Candidiasis was low (8%), 27% of patients harbored evidence of subclinical colonization by *Candida*. In addition, a significant correlation was found between *Candida*l colonization and low absolute lymphocyte counts. The significance of these findings in relation to systemic Candidiasis and rationale for therapy are discussed. **(Rodu B*et al.*, 1984)**.

Forty-six patients undergoing radiation therapy for oral/pharyngeal squamous cell carcinoma were evaluated clinically and by *Candidal* cultures before, during, and after irradiation. When salivary glands are included in the field of radiation, xerostomia occurs, causing progressive increases in oral *Candida* colonization. Because 17.4% developed clinical candidiasis during radiotherapy and the question of fungal resistance remains speculative, a recommendation for the prophylactic use of antifungal medication is unresolved **(Velia Ramirez-Amador*et al.*,1997)**.

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candida* species, the commonest being *Candida albicans*. The incidence varies depending on age and certain predisposing factors. There are three broad groupings consisting of acute candidiasis, chronic candidiasis, and angular cheilitis. Risk factors include impaired salivary gland function, drugs, dentures, high carbohydrate diet, and extremes of life, smoking, diabetes mellitus, Cushing's syndrome, malignancies, and immunosuppressive conditions. Management involves taking a history, an examination, and appropriate antifungal treatment with a few requiring samples to be taken for laboratory analysis. In certain high risk groups antifungal prophylaxis reduces the incidence and severity of infections. The prognosis is good in the great majority of cases (**AAkpan*et al.*, 2002)**.

A controlled study assessed the incidence of oral candidiasis, a xerostomia-related complication, in head and neck cancer patients receiving radiotherapy, with amifostine cytoprotection. Thirty-eight patients received 500 mg amifostinei.v. , prior to each radiotherapy fraction, while 16 patients received radiotherapy alone. Oral candidiasis was diagnosed according to the criteria described before. Subjective xerostomia scales were

completed by all patients. Mucositis was evaluated using the RTOG criteria. Oral candidiasis was diagnosed in 11/38 amifostine patients and in 9/16 controls (*P*=0.07). Severe xerostomia was reported by 4/38 amifostine patients and by 7/16 controls. Oral candidiasis was reduced with amifostine cytoprotection. Oral candidiasis is suggested as an objective, early, though indirect, endpoint for amifostine's radioprotective effect on salivary glands **(Ourania NG*et al.*, 2002)**

Twenty-seven consecutive patients receiving radiation to the head and neck were followed to assess risk factors for the development of candidiasis. One-third of the patients developed oral candidiasis during radiation therapy. Xerostomia was shown to correlate with risk of oropharyngeal infection (p = 0.033). The presence and use of oral prostheses were shown to correlate with oral colonization of *Candida albicans* before radiation therapy (p = 0.011). Alcohol use and smoking represent risk factors for oral colonization by *Candida* during radiation therapy (p = 0.023 and p = 0.045 respectively). These factors must be assessed in future studies of oropharyngeal candidiasis in radiation therapy **(Joel B. Epstein*et al.*, 1993).**

Another study related to head and neck cancer in patients receiving radiation therapy and Oral mucosal colonization and infection with *Candida* . Infection is marked by oral pain and/or burning and can lead to significant patient morbidity. The purpose of this study was to identify *Candida* strain diversity in this population by using a chromogenic medium, sub culturing, molecular typing, and antifungal susceptibility testing of clinical isolates. These results were then correlated with clinical outcome in patients treated with fluconazole for infection. Specimens from 30 patients receiving radiation therapy for head and neck cancer were cultured weekly for *Candida*. Patients exhibiting clinical infection were treated with oral fluconazole. All isolates were plated on CHROM agar *Candida* and RPMI medium, subcultured , and submitted for antifungal susceptibility testing and molecular typing. Infections occurred in 27% of the patients and were predominantly due to *Candida albicans* (78%). *Candida* carriage occurred in 73% of patients and at 51% of patient visits. Yeasts other than *C. albicans* predominated in carriage, as they were isolated from 59% of patients and at 52% of patient visits. All infections responded clinically, and all isolates were susceptible to

fluconazole. Molecular typing showed that most patients had similar strains throughout their radiation treatment. One patient, however, did show the acquisition of a new strain. With this high rate of infection (27%), prophylaxis to prevent infection should be evaluated for these patients (spencer *et al.* ,1999*).*

Candida albicans , is the major invasive fungal pathogen of humans, can cause both debilitating mucosal infections and fatal invasive infections. Understanding the complex nature of the host–pathogen interaction in each of these contexts is essential to developing desperately needed therapies to treat fungal infections. RNA-seq enables a systems-level understanding of infection by facilitating comprehensive analysis of transcriptomes from multiple species (e.g., host and pathogen) simultaneously. We used RNA-seq to characterize the transcriptomes of both *C. albicans* and human endothelial cells or oral epithelial cells during in vitro infection. Network analysis of the differentially expressed genes identified the activation of several signaling pathways that have not previously been associated with the host response to fungal pathogens. Using ansiRNA knockdown approach, we demonstrate that two of these pathways—platelet-derived growth factor BB (PDGF BB) and neural precursor-cell-expressed developmentally down-regulated protein 9 (NEDD9)—govern the host–pathogen interaction by regulating the uptake of *C. albicans* by host cells. Using RNA-seq analysis of a mouse model of hematogenously disseminated candidiasis (HDC) and episodes of vulvovaginal candidiasis (VVC) in humans, we found evidence that many of the same signaling pathways are activated during mucosal (VVC) and/or disseminated (HDC) infections in vivo. Our analyses have uncovered several signaling pathways at the interface between *C. albicans* and host cells in various contexts of infection, and suggest that PDGF BB and NEDD9 play important roles in this interaction. In addition, these data provide a valuable community resource for better understanding host-fungal pathogen interactions (yaoping*et al*., 2015).

Study was aimed to to detect Oral Candidiasis in children and adolescents with cancer and Identification of *Candida* spp in those Patients with malignant neoplastic disease that were receiving cytostatic treatment and had suspicious lesions of oral candidiasis.

Patients with antifungal therapy, active caries, calculus or intraoral appliances were excluded. A clinical evaluation was carried out. The lesion sample was taken and studied by direct exam and culture in CHROMagar-*Candida* and Sabouraud-Dextrose Agar with chloramphenicol. The identification of the isolated yeast was done by the filamentation test, carbohydrate fermentation and assimilation .The result showed that Most of the cases (69.35%) were positive to oral candidiasis, *C. albicans* was the most frequent species found, followed by *C. parapsilosis* (14.89%), *C. tropicalis* (12.77%), *C. krusei* (4.26%), *C. glabrata* (2.13%) and *C. lusitaniae* (2.13 %). In some cases more than one species were isolated (9.30%). The most frequent location of the lesion was in the tongue (72.70%). The pseudomembranous candidiasis was the most frequent clinical presentation found (78.71%). There were not significant statistically differences with regard to sex and age of the patient, type of neoplasic disease and cytostatic agent received. The results suggest that oral candidiasis is a frequent complication in the pediatric oncological population, being *C. albicans* the main etiological agent , however, there is an important participation of other *Candida* species (Haylen*et al*., 2007).

A study aimed to to investigate oral yeast carriage amongst patients with advanced cancer. Oral rinse samples were obtained from 120 subjects. Yeasts were isolated using Sabouraud's dextrose agar and CHROMagar™ *Candida*, and were identified using a combination of the API 20 C AUX yeast identification system, species-specific PCR and 26S r DNA gene sequencing. Oral yeast carriage was present in 66% of subjects. The frequency of isolation of individual species was: *Candida albicans*, 46%; *Candida glabrata*, 18%; *Candida dubliniensis*, 5%; others 5%. The increasing isolation of non-*Candida albicans* species is clinically important, since these species are often more resistant to antifungal drugs. Oral yeast carriage was associated with denture wearing ($P = 0.006$), and low stimulated whole salivary flow rate ($P = 0.009$). Identification of these risk factors offers new strategies for the prevention of oral *Candidosis* in this group of patients (A. N. Davies *et al., 2002).*

The aims of this systematic review were to determine, in patients receiving cancer therapy, the prevalence of clinical oral fungal infection and fungal colonization, to determine the impact on quality of life and cost of care, and to review current

management strategies for oral fungal infections .For all cancer treatments, the weighted prevalence of clinical oral fungal infection was found to be 7.5% pre-treatment, 39.1% during treatment, and 32.6% after the end of cancer therapy. Head and neck radiotherapy and chemotherapy were each independently associated with a significantly increased risk for oral fungal infection. For all cancer treatments, the prevalence of oral colonization with fungal organisms was 48.2% before treatment, 72.2% during treatment, and 70.1% after treatment. The prophylactic use of fluconazole during cancer therapy resulted in a prevalence of clinical fungal infection of 1.9%. No information specific to oral fungal infections was found on quality of life or cost of care. The conclusions that there is an increased risk of clinically significant oral fungal infection during cancer therapy. Systemic antifungals are effective in the prevention of clinical oral fungal infection in patients receiving cancer therapy. Currently available topical antifungal agents are less efficacious, suggesting a need for better topical agents (Rajesh*et al*.,2010).

CHROMagar *Candida* is a recently described and differential medium for the isolation and the presumptive identification of clinically important yeasts. We evaluated it with 262 yeast strains from clinical specimens, including 173 *Candida albicans,* 21 *Candida tropicalis,* 8 *Candida krusei,* 49 *Candida glabrata,* and 12 strains of other yeast species. Strains were presumptively identified on the basis of colony color and texture. These observations were compared with conventional identification results. *Candida albicans* was identified correctly in 170 (98%) of the 173 strains. A total of 46 of the 205 specimens that were plated on CHROMagar contained mixed cultures of yeast. Thirty-seven (80%) of these mixed cultures were not detected in the original specimens. CHROMagar *Candida* was useful for the rapid presumptive identification of *Candida albicans* and facilitated the recognition of mixed cultures. For other yeast species, it may provide additional information to laboratories that do not regularly perform identifications beyond the germ tube test (Heather L. Powell*et al*., 1998).

A fungal infection represents a growing problem in children with hematologic malignancies, during chemotherapy induced neutropenia. Fungal colonization is considered a major risk factor for subsequent fungal infections. The aim of this retrospective study was to evaluate prevalence of fungal infection among children

admitted to hospital between 2005 and 2010 in Tehran, Iran. 617 hematological patients in the age range of neoteric to 19 years old were enrolled and 87 cases with invasive fungal infections were extracted from patients' files and documented. Diagnosis of fungal infections was based on the local biopsy and pathology for mucormycosis, blood culture, urine culture and clinical examination for *Candidasis* and galactomannan for aspergillus. The majority of the infections were caused by candidia spp (74.7%). Among candidiasis patients, oral infection had the highest manifestation (92.3%). There was a significant association between mortality and the type of fungal infection ($p < 0.0001$). Our finding suggests that there is a high rate of fungal infections in children receiving remission therapy for onco-hematology. These results help improve the management of these patients, however Further studies are needed (Ansari Sh*et al., 2015).*

There is an interest in the association between oral cancer risk and *Candida*-associated promotion of mucosal dysplasia continues. However, little is known of the presence and amount of oral yeast in the mouths of healthy patients without mucosal lesions. The purpose of this prospective cross-sectional clinical study was to ascertain the prevalence and degree of carriage of *Candida* in the oral cavities of a non-cancer population, with reference to a range of parameters affecting the oral environment. Oral rinse samples were collected from a sample of 203 patients attending the Royal Dental Hospital of Melbourne and analysed for the presence and degree of colonization of yeast species that were phenotypically identified as albicans and non-albicans species. Oral yeast carriage was found in 98/203 patients (48.3%), and of these, 83 (84.7%) patients carried *C. albicans.* There was no statistical difference in carriage when comparing gender, age, or presence of a removable prosthesis. Both smoking and the presence of active carious lesions were found to be positively correlated with the carriage of oral *Candida*. Individuals who are current smokers are nearly seven times more likely to have oral *Candida*, and participants with high *Candida* colonisation are more likely to be current smokers. Participants with active carious lesions were also more likely to carry oral *Candida* (Mun MS *et al*., 2015).

Oral colonization of *Candida* could lead to later development of oropharyngeal candidiasis or candidemia among the immunocompromised patients. This study aims to

describe the occurrence and risk factors of oral *Candida* colonization in patients with malignancies. The differences of risk factors for oral *Candida* colonization in patients with different cancers require different strategies for the prevention and control of *Candida* infection. Old aged patients with pulmonary cancer and digestive tract malignant tumor are high-risk population for *Candida* colonization. Increasing frequency of teeth brush might be helpful for preventing *Candida* colonization (Sun H *et al*., 2015).

Chapter Three

Materials and Methods

Chapter Three

MATERIALS AND METHODS

3.1 Study design

3.1.1 Study type

The study was cross-sectional and hospital based study.

3.1.2 Study area

This study was carried out in Khartoum state at Radiation and Isotopes Khartoum centre (El Zara Hospital).

3.1.3 Study Duration

The study was conducted from May to July 2015.

3.1.4 Study Population

3.1.4.1 Inclusion Criteria

Known Sudanese cancer patients receiving cancer treatment with different age and sex were included.

3.1.4.2 Exclusion criteria

Untreated cancers patients were excluded and those who were received antifungal treatment.

3.2 Data Collection

After explaining the purpose of the study, data were collected from each subject by interviewing check list tool (appendix). The data included information such as name ,age, sex, tribe ,residence place, cancer type, type of cancer treatment (chemotherapy, Radiation or Both), when cancer first diagnosed, chronic disease if found(Diabetes

mellitus, Blood pressure, any respiratory issues e.g.(asthma, tuberculosis ,smoking) (duration) and snuff (duration).

3.3. Sample Size

One hundred oral swabs were taken.

3.4 Specimen collection and preparation

Sterile disposable wool-cotton swabs were used. Oral swab was collected by asking patients to open their mouths, swab was opened, then swab was rotated from the inner part of mouth cheeks, then swab pressed for up to two minutes in the inner part of mouth cheeks, then recapped and cultured immediately.

3.5 Laboratory Examination

The specimens were cultured on to Sabouraud's dextrose agar media supplemented with chloramphenicol, incubated at 37c for an overnight .Identification of *Candida* was carried out microscopically after staining with Gram's stain , methylene blue ,culturing the isolated organism in human serum to detect the germ tube and by culturing it on *Candida* chromogenic agar culture media**.**

3.5.1 Sabouraud's dextrose agar:

All oral swabs were cultured in Sabouraud's dextrose agar media supplemented with chloramphenicol antimicrobial agent to exclude the normal flora of the oral cavity, incubated at 37c for 24-48 hours

Result

A creamy, small-moderate, slightly smooth colonies with a yeasty odour were isolated.

3.5.2. Gram's stain:

After sample had cultured in Sabouraoud's dextrose agar, a smear prepared by taking a part of colony and then added to a small drop of normal saline placed in a clean, dry glass slide, mix gently, wait until dry, fix with heat, and then stain with Gram's stain as follow:

1-Add the basic stain which it is crystal violet in to slide smear, and then wash after 1 minute.

2-Add loguol iodine to the smear slide and wash after 1 minute.

3-Add alcohol to the smear and wash after 15 seconds.

4-Add counter stain in which it is saffranine which stain the smear with red colour in case of Gram negative.

5-Examine the slide under the microscope, add oil drop and use ×100 the oil lens.

Result

Gram positive yeast as *Candida* yeast takes the violet colour of crystal violet stain, different in size (oval-round to elongate).

3.5.3. Methylene blue

A smear prepared by taking a part of a single colony then transfer it to a clean slide contained a small drop of normal saline ,mix gently ,wait to dry ,fixed with heat ,then flood slide with methylene blue stain and wash it after 1 minute with tap water.

Result:

Blue yeast with different size and shape ovoid, elongated due to different strain .

3.5.4. Germ tube test

A small amount of the yeast colony was inoculated in to 0.5 ml of human blood serum, incubated at 37-36c for up to 3 hours. After incubation wet preparation was examined microscopically with x40 magnification. A small drop of suspension was placed in clean dry slide, covered with cover glass to Look for the characteristic hyphal elements.

Results

The presence of germ tubes having no constriction at the site of origin is considered a positive test result, consistent with *C.albicans*. However the absence of germ tubes does not rule out *C.albicans.*

3.5.5 Chromogenic *Candida* Agar culture media

Ii is a differential media which differentiate between *Candida* species strains by giving different colour crosspond to each strain according to manufacture .In this study the media that used is from Himedia . A after *Candida* species is isolated in Sabouraud's dextrose agar which supplemented with chloramphenicol antibiotic 2 cc for every 1000 ml of distilled water , 2-3 colonies was taken by a sterile wire loop and sub cultured in *Candida* chromogenic agar media ,Incubated at 36-37c for 24-48 hours. The result is according to manufacture as follow:

-Blue –dark green colonies it mean that the isolate is *Candida tropicalis.*

-Light green colonies for *Candida albicans.*

-Creamy-light violet colour, the isolate is *Candida krusei.*

Chapter Four

Results

Chapter Four

Result

4.1. Result

The individuals in this study were males and females, adults with an age ranging from 18 years old to 83 and children ranging from 2years to 17 years old.Fig:1.

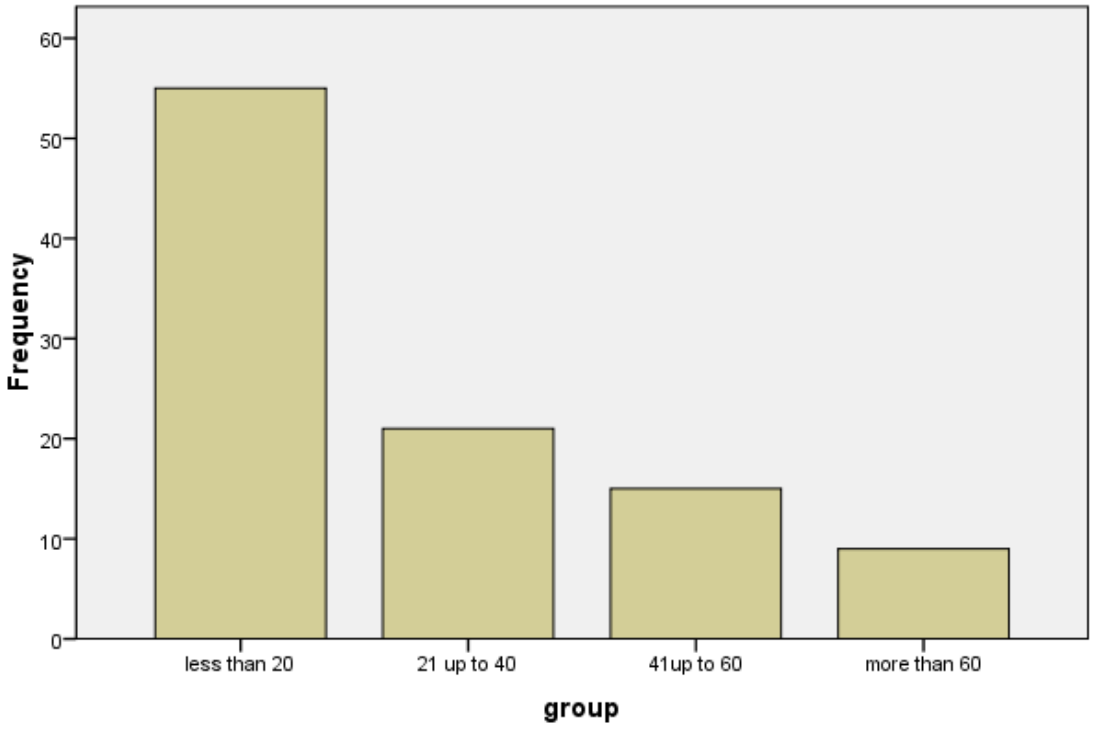

Figure (1): Frequency of age group

Hudered oral swabs were collected from the oral cavity of Sudanese cancer patients who were received treatments in Radiation and Isotopes centre of Khartoum (al Zarra Hospital) in order to detect the prevalence of an oral Candidiasis among them. Forty percent of those patients were affected with an oral Candidiasis and sixty percent of them were not affected (Table 1).

Table 4.1 Prevalence of oral Candidiasis among cancer patients

Oral Candidiasis	**Number**	**Percent**
Positive	40	40%
Negative	60	60%
Total	100	100%

This table showed that *Candida albicans* were the most prevelance species among all age groups .The most affected age group with an oral Candidiasis were those less than 20 years old (Table 2).

Table 4.2 Age group and *Candida* species strain distribution.

Age Group	***C. albicans***	***C.tropicalis***	***C. krusei***	**NO GROWTH**	**Total**
Less than 20	19 (67.9 %)	4 (50%)	0 (0%)	32 (53.3%)	55 (55%)
21 up to 40	2(7.1%)	3 (37.5%)	2 (50%)	14 (23.3%)	21 (21%)
41up to 60	4 (14.3%)	1 (12.5%)	2 (50%)	8 (13.3%)	15 (15%)
more than 60	3 (10.7%)	0 (0%)	0 (0%)	6 (10%)	9 (9%)
Total	28	8	4	60	100 (100%)

Table showed that 40 % of Cancer patient get an oral candidiasis and showed a growth in Sabouraoud's dextrose agar supplemented with chloramphenicol antibiotic .while 60% of them are not showed a growth (Table 3).

Table 4.3 *Candida* growth in Sabouraoud's dextrose agar.

Growth on sabouroud,s dextrose supplemented with chloramphenicol	***C.albicans***	***C.tropicalis***	***C.kruzei***	**No growth**	**Total**
Growth	28 (100%)	8 (100%)	4 (100%)	1 (1.7%)	41 (41%)
No growth	0 (100%)	0 (0%)	0 (0%)	59 (98.3%)	59 (59%)

Table showed that 40 % of Cancer patient get an oral candidiasis and showed a growth in Sabouraoud's dextrose agar supplemented with chloramphenicol antibiotic .while 60% of them are not showed a growth (Table 3).

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Growth	28 (100%)	8 (100%)	4 (100%)	1 (1.7%)	41 (41%)
No growth	0 (100%)	0 (0%)	0 (0%)	59 (98.3%)	59 (59%)
Total	28	8	4	60	100 (100%)

This table showed that oral Candidiasis was affected cancer patients with 40%. Strain of *Candida* species was differentiated by chromogenic *Candida* agar media. *C .albicans* was the most prevalence species strain with 28% which gave light green colonies, blue colonies for *C .tropicalis* which was 8% and the creamy-moove colonies for *C. krusei* were 4% (Table 4).See figure (2)

Table 4.4 Growth of *Candida* species in *Candida* chromogenic agar media

	Growth on *Candida* Chromogenic Agar	***C.albicans***	***C.tropicalis***	***C.kruzei***	**No growth**	**Total**
	GREEN	28 (100%)	0 (0%)	0 (0%)	0 (0%)	28 (28%)
	BLUE	0 (0%)	8 (100%)	0 (0%)	0 (0%)	8 (8%)
	CREAMY-MOOVE	0 (0%)	0 (0%)	4 (100%)	0 (0%)	4 (4%)
	NO GROWTH	0 (0%)	0 (0%)	0 (0%)	60 (100%)	60 (60%)
Total		28	8	4	60	100 (100%)

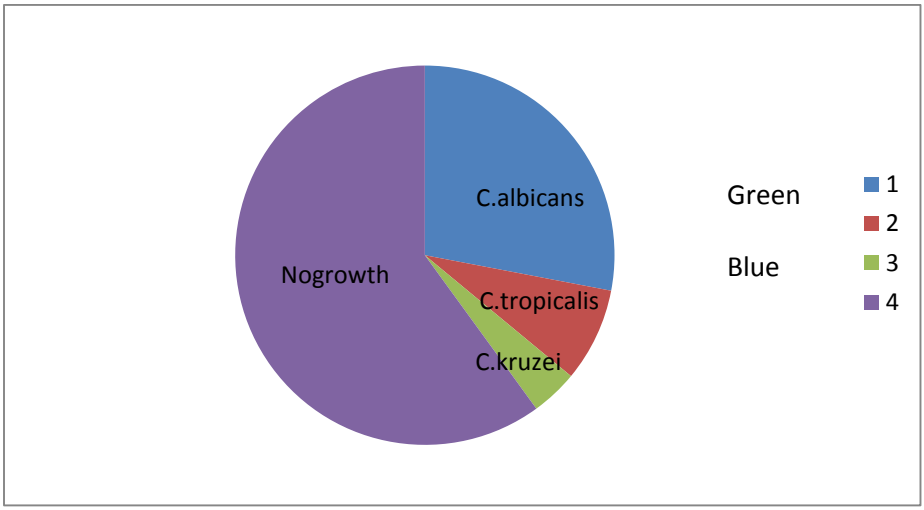

Figure (2): Identification of *Candida* species in chromogenic *Candida* agar media

This table showed that male was most affected with an oral Candidiasis with 23%.Female were less affected with 17% (Table 5).See figure (3).

Table 4.5 Distribution of oral candidiasis among sex.

Sex	***C. albicans***	***C .tropicalis***	***C. krusei***	**NO growth**	**Total**
Male	18 (64.3%)	4 (50%)	1 (25%)	43 (71.7%)	66 (66%)
Female	10 (35.7%)	4 (50%)	3 (.25%)	17 (28.3%)	34 (34%)
Total	28	8	4	60	100

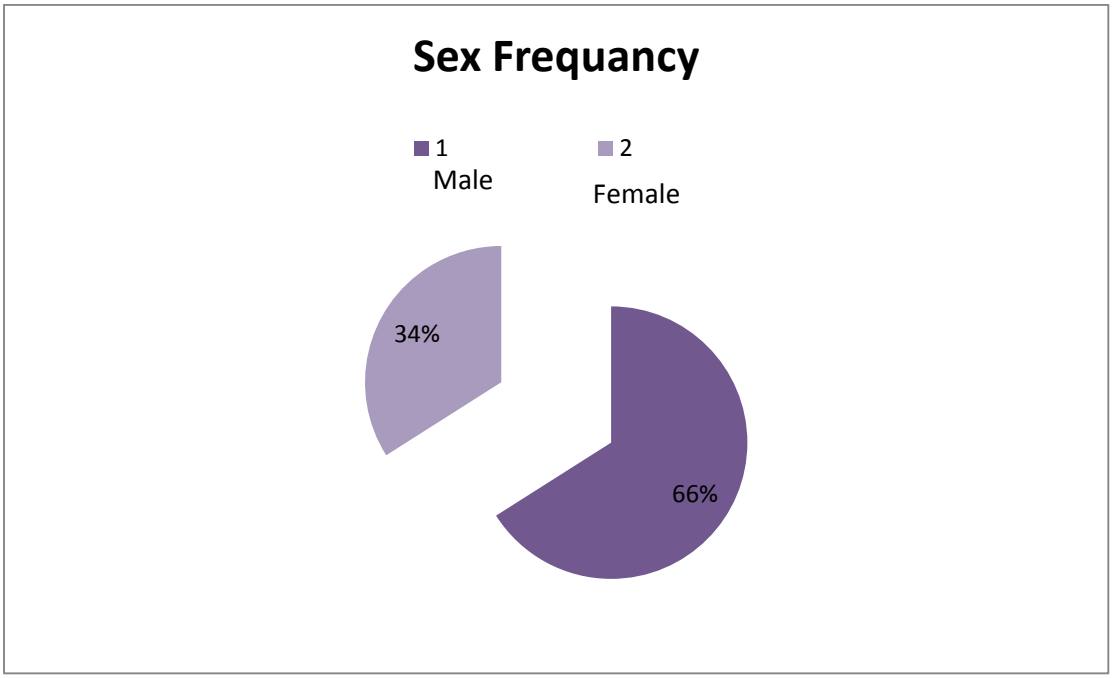

Figure (3): Sex Frequency

This table showed that the high prevalence range of an oral Candidiasis were in lukemic cancer patients with 35 % . *Candida albicans* was the most prevalence strain a in this cancer type (Table 6). See figure (4).

Table 4.6 Distribution of oral Candidiasis among the type of cancer

Cancer Type	***C. albicans***	***C.tropicalis***	***C. krusei***	**NO GROWTH**	**Total**
RS	1 (3.6%)	0 (0%)	1 (25%)	4 (7%)	6 (6%)
GIT	2 (7.1%)	2 (25%)	0 (0%)	7 (11.6%)	11 (11%)
GUT	5 (17.9%)	1 (12.5%)	2 (50%)	12 (20%)	20 (20%)
Skin	0 (0%)	0 (0%)	0 (0%)	2 (3.3%)	2 (2%)
Brain	1 (3.6%)	0 (0%)	1 (25%)	0 (0%)	2 (2%)
Leukemia	13 (46.4%)	4 (50%)	0 (0%)	18 (30%)	35 (35%)
Lymphoma	5 (17.9%)	1 (12.5%)	0 (0%)	13 (21.7%)	19 (19%)
Bone	1 (3.6%)	0 (0%)	0 (0%)	4 (6.7%)	5 (5%)
Total	28	8	4	60	100

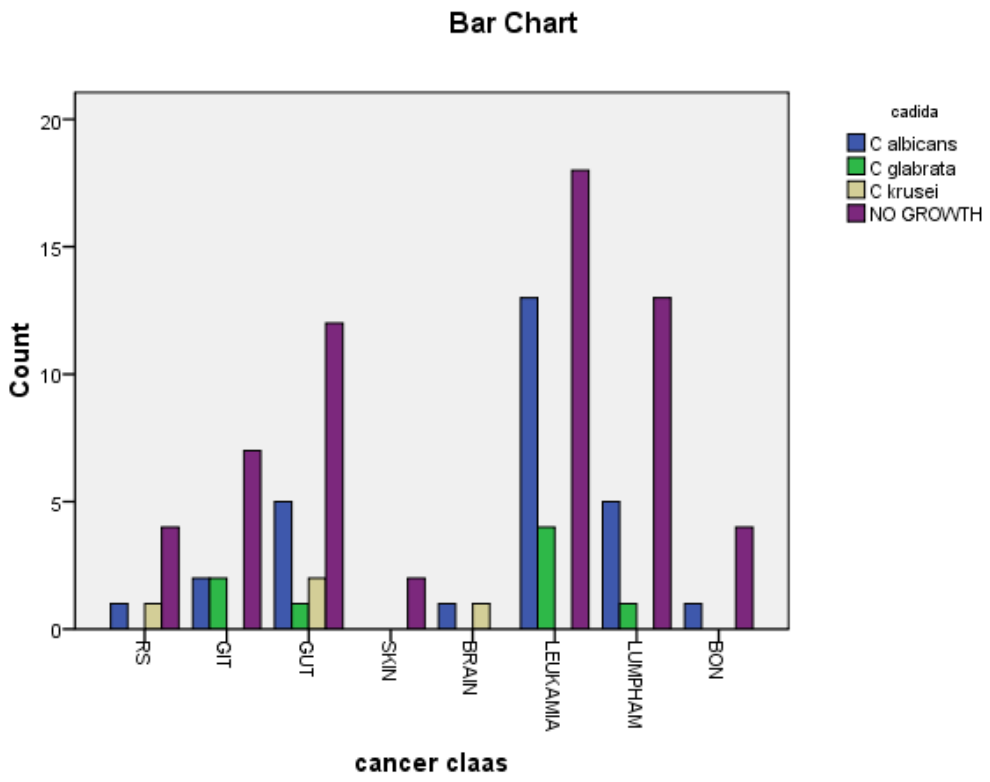

Figure (4): Distribution of *Candida* Strains among Cancer Types

RS=Respiratory System

GIT=Gastro Intestinal Tract

GUT=Genital Urinary Tract

Chapter Five

Discussion

Chapter Five

Discussion

5.1. Discussion

The prevalence of an oral Candidiasis obtained in this study among 100 Sudanese cancer patients with different cancer types, who were received different cancer treatment including chemotherapy, radiation or both who were treated in Radio Isotopes Centre of Khartoum (al Zarra hospital) was 40% this incidence is not that much highly increased, although these patients were immune suppressed .This result is similar to **(Rodu B*et al.*, 1984)**.60% of those patients were not affected with an oral candidiasis and showed no growth in Sabouraoud's dextrose agar media supplemented with chloramphenicol .Growth of *Candida* in Sabouraoud's dextrose agar is significant P=0.000 it is less than 0.05

Study showed that there was significant relationship between oral Candidiasis and chromogenic agar media in which p=0.000 which was less than the p.Value 0.05.

From these patients within an age group less than 20 years old were infected with 23%, patients within an age group range between 21-41 years old were 7%, patients within an age group of 41-60 years old were affected with an oral Candidiasis with 7% and those who were with an age more than 60 years old *were affected with 3%* compare with other studies.

From those 100 patients 23% of them were male get an oral Candidiasis,while female are less infected with 17% of an oral candidiasis .P=0.187 it is insignificant relation between oral candidiasis and and sex.

The most prevalence *Candida* species strain was *Candida albicans* with percentage 28%, followed *by Candida tropicalis* 8% and *Candida kruzei* 4%.

The prevalence of an oral Candidiasis according to locality resident place of those cancer patients who were use cancer treatment showed that Omdurman city of Khartoum

state get the high frequency of an oral Candidiasis this may due to low hygiene in rural area in Omdurman city and low economic condition.

In this study cancer patients with different cancer types included Respiratory system (Nasopharyngeal carcinoma, Pyriform fossa , Lung cancer),Genital urinary tract which included (Neuroblastoma ,Bladder ,Prostate ,Cervix ,Rectum ,Ano-Rectum) ,Gastro intestinal tract (Stomach ,Colon , Colo-Rectum ,Liver),skin(Melanoma),Cerebral Spinal System cancer (Brain) ,Lymphatic system which involved (non Hodgkin's lymphoma-Burkitt's lymphoma-Hodgkin's lymphoma),leukaemia which included (ALL,AML,CML) and bone and connective tissues which involved (Osteosarcoma ,Rhabdomyosarcoma).Study showed that there is no significant relationship between oral Candidiasis in which P=0.178.

.Also Gail tribe showed more frequency of an oral Candidiasis this might be due to genetic or ethnic behaviour.

5.2. Conclusion

The result of this study concluded that:

The prevalence of an oral Candidiasis in cancer patients who were received treatment was 40%.

The most prevalence strain is *Candida albicans* with 28% which followed by *Candida tropicalis* 8% and *Candida kruzei* 4%.

Patient in early age less than 20 years old were more affected with an oral Candidiasis

The most affected cancer patients were those who are diagnose as a leukemic patients.

Cancer patients Resident in Omdurman city are showed high frequency of an oral Candidiasis

Cancer patients from Galian tribes were most affected with an oral Candidiasis than others.

5.3. Recommendation

Oral swab must take and cultured every treatment cycle.

Dry mouth which seen in most cancer patient must solve out by drinking water and pay an attention that dry mouth itself can lead to oral Candidiasis.

All finding cases must treat and followed up after treatment.

References

References

1. **Akpan A and Morgan R (2002) .**Oral candidiasis. Postgraduate Medical Journal 78; (922):455-459.
2. **Davies N A, Brailsford S, Broadley K and Beighton D (2002)**''Oral yeast carriage in patients with advanced cancer''*wiley online library ,Oral microbiology and immunology* 17;(2):79-84.
3. **Ansari Sh, Elahi M and Shirzadi E (2015).**The Prevalence of fungal infections in children with hematologic malignancy in Ali-Asghar Children Hospital between 2005 and 2010.Iranian *journal of pediatrics hematology and oncology* 5;(1):1-10.
4. **Mims.C, Dockrell M H, Goering V R, Roitt I, Wakeline D and Zuckerman .M (2004)**Medical microbiology'' Updated 3rd ed,p231.
5. **Schiff D ,Kesari S and Patrick Y. Wen (2008)** Cancer Neurology of Clinical Practice: Neurologic Complications of Cancer and Its Treatment.p499.
6. **Heymann D (2009)** *Bone Cancer progression and Therapeutic Approaches* 1sted.p193.
7. **Gravina G H, Morán G E, Zambrano O, Chourio L M, Valero R S, Robertis S and Mesa L (2007)**.Oral Candidiasis in children and adolescents with cancer Identification of *Candida* spp. *Medicina oral ,pathologia oral YCirugi a Bucal* (internet)12.6 .
8. **Heather L. Powell, Crystal A. Sand and Robert P. Rennie (1998)**.Evaluation of CHROMagar *Candida* for presumptive identification of clinically important *Candida* species. *Diagnostic microbiology and infectious disease journal* 32;(3):201-204.
9. **Hare.J (2008).**Sabouraud agar for fungal growth protocols .American society for microbiology library.
10. **Joel B. Epstein,Marshall M. Freilich and Nhu D. Le (1993).**Risk factors for oropharyngeal candidiasis in patients who receive radiation therapy for malignant conditions of the head and neck Oral surgery, Oral medicine, Oral pathology journal 76;(2):169-174.
11. **JuiletV.Spencer (2009)***Cervical Cancer*.p10-11

12. Burket.W.L, Glick M and Greenberg.S.M (2008). Burkets Oral Medicine**.**p79.

13. **Taha M (2011)** Medical Mycology Atlas of Medically Important Fungi and Dermatomycosis 1st ed. P24-36.

14. **Mun MS[1], Yap T, Alnuaimi AD, Adams GG and McCullough MJ (2015).**Oral *Candida* l carriage in asymptomatic patients. *Australian dental journal*.

15. **Anastasia SL, Aristea V, Georgia K,George P, Konstantinos D, Konstantinos K ,Ioannis P ,Ioannis Y , Ourania NG, Vassilis K (2003).**Oral candidiasis in head and neck cancer patients receiving radiotherapy with amifostine cyto protection. Oral Oncology journal 39;(4):397-401.

16. **Engelkirk GP and Engelkirk D L J (2008) .**Laboratory diagnosis of infectious disease: Essential of diagnostic microbiology.

17. **Kumar P and Clark M (2005)***Clinical Medicine* 6thed ,P 500-523

18. **Rajesh V. Lalla,Marie C. Latortue**, **atherine H. Hong**,**Anura Ariyawardana, Sandra D'Amato-Palumbo,Dena J. Fischered and *et al* (2010)**.A systematic review of oral fungal infections in patients receiving cancer *therapy. Sportive care in cancer journal* 18;(2):985-992.

19. **Raymond W R (2007)** *Cancer Biology* 4thed ,p4.

20. **Robert Wilkins (2011)** Oxford Handbook of Medical Science, p880 chapter 13.

21. **Rodu B, GriffinIL,Gockerman JP (1984).**Oral *Candida*sis in cancer patients. *Southern Medical Journal;* 77(3):312-314.

22. **Spencer W Redding,Richard C. Zellars, William R. Kirkpatrick, Robert K. McAtee, Marta A. Caceres, Annette W. Fothergill, Jose L. Lopez-Ribot, Cliff W. Bailey, Michael G. Rinaldi, and Thomas F. Patterson (1999).**Epidemiology of oropharyngeal *Candida* colonization and infection in patients receiving radiation for head and neck cancer. Clinical microbiology journal 37 ;(12):3896-3900.

23. **Sun H, Chen Y, Zou X, Li H, Yin X, Qin H, Liu R, Yu C, Li Q, Yu K, Han X, Zou J, Ge C, Han L (2015)**.Occurrence of oral *Candida* colonization and its risk factors among patients with malignancies in China. Clinical oral investigation. pubmed .

24. Price T, Montgomery P, Birchall M, Gullane P (2012) A Diagnostic Atlas of Tumors of the Upper Aero-Digestive Tract: A Transnasal Video Endoscopic Approach.p42.

25. **Amador R V, Mayer P, Sol Silverman JR, , Tyler M , Quivey J (1997).** *Candida* l colonization and oral Candidiasis in patients undergoing oral and pharyngeal radiation therapy.84.2:149-153. *Oral surgery, oral medicine, oral pathology and oral radiology Journal.*

26. Vincent T. DeVita, Theodore S. Lawrence and Steven A . Rosenberg volume 2 (2008) DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology volume 2 .chapter 44.2 p 1670 .

27. **Yaoping Liu, Amol C. Shetty,Jennifer A. Schwartz, L. Latey Bradford , WenjieXu, Qyunh T. Phan, PritiKumari, AnupMahurkar, Aaron P. Mitchell, Jacques Ravel, Claire M. Fraser, Scott G. Filler and Vincent M. Bruno(2015).**New signaling pathways govering the host response to *Candida albican* infection in various nashies.pub med 25;(5):679-689.

Appendices

APPENDICES

Appendix (1)

1.1. Equipment's

Incubator	memert	Germany	Model 500
Incubator	SuperFit	India	Model R500/02
Pharmacy Refrigerator		China	Model YY-120
Oven			
Autoclave		China	Model LS-50Hs
Microscope	Olympus	Germany	
Sensitive balance			

Appendix (2)

Reagents:

Distilled water

Human serum

Gram's Stain Kit Crescent Diagnosis 250 ml

Methylene Blue Crescent Diagnosis 250 ml

Chromogenic Agar Media for *Candida* Himedia India

Sabourauoud's Dextrose Agar Himedia India

Normal saline

Oil for 100 × immersion lens

Appendix (3)

Other materials:

Vacuumed tube plain containers5ml GEETMED

Cotton

Pasteur Pipette

Disposable Gloves

Disposable Cotton-Wool Swab

Wooden stick

Cover glass

Slide glass

Appendix (IV)

Sudan University of Science and Technology

College of Graduate Studies

Check list

Date:

Name:

Age: **Resident place:**

Sex: **Tribe:**

Type of cancer:

When first diagnose with a cancer:

Type of cancer treatment:

Number of treatment cycle:

Chronic disease: {Diabetes mellitus (__)-blood pressure (__)}

Respiratory problem or disease :{ Asthma (__)-Tuberculosis (__)}

Bad habits:

1-Snuff(__) .Duration of snuff(_____)

2-Smoking (__) .Duration of smoking (_____)