بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Frequency of *Candida* species among Diabetic Patients with Urinary Tract Infection at Advanced Diagnostic Center – Khartoum North

تكرار عدوى أنواع الكانديدا عند مرضى السكري الذين يعانون من التهاب المسالك البولية في المركز التشخيصي المتطور -الخرطوم شمال

A dissertation Submitted In Partial Fulfillment of the Requirements of M.Sc. Degree in Medical Laboratory Sciences (Microbiology)

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2018

الآيـــــة

قال تعالى:

(وَالْبَلَدُ الطَّيِّبُ يَخْرُجُ نَبَاتُهُ بِإِذْنِ رَبِّهِ وَالَّذِي خَبُثَ لَا يَخْرُجُ إِلَّا نَكِدًا كَذَلِكَ نُصَرِّفُ الْآيَاتِ لِقَوْمٍ يَشْكُرُونَ)

صدق الله العظيم

سورة الاعراف (الآية - 58)

Dedication

I dedicate this study to

My father the source of superstitious,

Who have made it possible, those who always been on my side,

My mother, my brothers

Whom I feel about them with beautiful emotion

To them all I dedicate this study as a sign

of thanks.

Acknowledgement

Thank to almighty Allah for helping me to complete this work.

I would like to convey my gratitude to **Dr.Wafa Ibrhim Elhag** for her supervising guide help. A lot thanks are extended to the Advanced Diagnostic Center Khartoum for helping me in specimen collection.

I would like to express my thankfulness to Sudan University research center, also Microbiology Department, Sudan University of Science and Technology.

Abstract

candiduria more common in patients with indwelling urinary catheters, systemic antibiotic use, previous surgery and diabetes mellitus. Most cases are asymptomatic and require no treatment. Progression to candiedmia is rare unless patients are at high risk for invasive disease. This study was conducted in the Advanced Diagnostic Center Khartoum North during the period from March to June 2017 to detect frequency of *Candida* species that associated with urinary tract infection among diabetic patients.

Two hundreds urine samples were collected from diabetic patients (type 1 and type 2). One hundreds of them have symptoms of UTI and other one hundreds had not. Their age ranged from 21 to 80 years with 52.21 mean, 88 were males and 122 were females. All urine samples were directly examined using standard microbiological technique.

The results revealed that the frequency of *Candida* species infection is more common in symptomatic patients 4(4%) than asymptomatic patients 1(1%) and in females 3(60%) more than males 2(40%). In addition, statistically there were no correlation between cadiduria infections and age, duration of disease, and gender of diabetes mellitus patients.

According to this study the frequency *Candida* species infection among diabetes mellitus patients was low.

Further studies are recommended with molecular techniques and large sample size to validate this result.

المستخلص

"الكانديدايوريا" أكثر شيوعا عند المرضى الذين يعانون من القسطرة البولية المستقرة، واستخدام المضادات الحيوية الجهازية، والجراحة السابقة والسكري. معظم الحالات تكون بدون أعراض ولا يعاد علاجها. التطور الي "الكانديديميا" نادر ما لم يكن المرضى معرضين للخطر الشديد للأمراض الانتهازية. أجري هذا البحث في المركز المتطور الحديث في الخرطوم شمال خلال الفترة من مارس إلى يونيو 2017 للكشف عن تكرار أنواع فطريات "الكانديدا" التي ترتبط مع التهاب المسالك البولية بين مرضى السكري.

تم أخذ مئتان عينة من المرضى الذين سبق تشخيصهم بمرض السكري (النوع 1 والنوع 2). مائه منهم لديهم أعراض التهاب المسالك البولية ومائه اخرون ليس لديهم اي اعراض، تراوحت اعمارهم من 21 الي 80 سنه مع متوسط اعمار 52.21 سنه ، 88 منهم كانوا رجال و122 كانوا نساء. تم فحص جميع عينات البول مباشرة باستخدام التقنيات الميكروبيولوجيه الصحيحة.

أظهرت النتائج أن انتشار عدوى فطريات "الكانديدا" هو أكثر شيوعا في المرضى الذين يعانون من أعراض التهاب المسالك البولية 4(4%) من الذين لا يعانون من الأعراض 1(1%). وفي الإناث 3(60%) أكثر من الذكور2(40%) . وبالإضافة إلى ذلك، وجد البحث أنه لا يوجد ارتباط بين عدوي فطريات "الكانديدا" حسب العمر، ومدة المرض والنوع من مرضى السكري.

حسب هذه الدراسة تكرار عدوي أنواع فطريات "الكانديدا" منخفضه لدي مرضي السكري. يفضل في الدراسات المستقبلية استخدام التقنيات الجزيئة وعدد كبير من العينات للتحقق من صحة هذه النتيجة.

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Chapter One
Introduction

1. Introduction, Rationale and Objectives

1.1. Introduction

More than 100.000 species of fungi have been recognized and described. However, less than 500 of these species have been associated with human diseases, and no more than 100 are capable of causing infection in otherwise normal individuals (William *et al.,* 2003). The remainders are only able to produce disease in hosts that are debilitated or immune compromised (William *et al.,* 2003).

Candidiasis is the most common fungal disease in humans, affecting the skin, mucosa, and various internal organs, caused by *Candida*, a yeast like fungus that produces pseudoyhphae (Apurba and Sandhya., 2016).

Infection of (UTI) due to *Candida* is uncommon because the significance of systemic factor in the defect of urinary tract against *Candida* infection. Secretion from the prostate gland in men and from the preiurethral gland in women have been reported to be fungistatic in addition growth *Candida* at site on mucous may be suppressed by other normal flora. (Fisher *et al.,* 1982). Predisposing factors that are associated with increased risk of infection with Candiduria include physiological state (e.g extremes of age and pregnancy), low immunity "e.g patients on steroid or immunosuppressive drugs, HIV, malignancy"patient on broad-spectrum antibiotic and diabetes mellitus (Fisher *et al.,* 1982).

Diabetes mellitus (DM) is a group of metabolic diseases characterized by high blood sugar resulting from defects in insulin secretion, insulin action, or both. Nearly 90% of patients with DM have Type 2 diabetes (Gougeon *et al.,* 2006).

Type 1 DM is absolute insulin deficiency in other words, the body cannot produce insulin. This is from a defect in the pancreas, the insulin-

producing organ of the body. Type 1 DM is often understood to be an auto-immune disorder, in which the body destroys its own tissue (in this case, the cells of the pancreas) (Gougeon *et al.*, 2006).

Type 2 DM arises because of insulin resistance. In this case, the body may be producing insulin but cannot use it properly. These individuals usually carry excess body fat; 80% of clients with Type 2 DM are obese at onset (Gougeon *et al.*, 2006). The world health organization (WHO), Recognizes three main forms of diabetes mellitus, type 1, type 2, and gestational diabetes mellitus (Rother., 2007).

Patients with diabetes mellitus (DM) are more prone to develop infections compared with the general population (Shah and Hux., 2003), with urinary tract infections (UTIs) being among the most commonly encountered (Schappert., 1999).

It has been estimated that UTIs account for 7 million hospital visits per year, along with 1 million visits to the emergency department, in the United States, (Schappert., 1999). Involving an estimated annual cost of ~US≥1.6 billion to the health care system (Foxman., 2002).

Several factors contribute to an increased infection risk in patients with DM: defects in the host immune defense mechanisms (such as impaired neutrophil function, decreased T-cell-mediated immune response, low levels of prostaglandin E, thromboxane B2, leukotriene B4), (Teodora *et al* ., 2017), incomplete bladder emptying due to autonomic neuropathy, and poor metabolic control (Fünfstück *et al.*, 2012). A higher glucose concentration in the urine allows urinary colonization by pathogenic microorganisms (Teodora *et al*., 2017).

Various types of UTIs may develop in patients with DM: asymptomatic bacteriuria (ASB), lower UTI (cystitis), upper UTI (pyelonephritis), and severe urosepsis, requiring careful management in this category of patient), (Teodora *et al.*, 2017). Rare complications of UTIs, including

renal papillary necrosis, emphysematous cystitis and pyelonephritis, and intrarenal and perinephric abscesses, occur more often and with greater severity in patients with DM), (Teodora *et al*., 2017).

Escherichia coli and other *Enterobacteriaceae* are the most commonly isolated uropathogens both in females and in males with DM, similar to the general population. However, UTIs caused by unusual and antibiotic-resistant uropathogens as well as fungal UTIs appear to be more frequently reported among patients with DM (Bonadio *et al.*, 2006).

Lower urinary tract candidiasis is usually the result of a retrograde infection, while renal parenchymal infection most often follows candidemia. In addition to asymptomatic Candiduria, recognized clinical forms of Candidal urinary tract infections include bladder infection, renal parenchymal Candidal infection, and infections associated with fungus ball formation (Fisher *et al.*, 1982).

1.2. Rationale

Candida species are the most frequently recovered fungi from genital and urinary tract. Candiduria, which means the presence of *Candida* in urine, it caused by *Candida albicans* and *Candida non-albicans* species of the genus *Candida*. Identification of *Candida* isolates to the species level is required due to changes in dominant species and different antifungal susceptibility patterns observed in recent years (Yashavanth *et al*., 2013).

Broad- spectrum antibiotic therapy, application of catheters, endocrine disorders such as diabetes mellitus, prolonged hospitalization, primary or secondary weakness in immune system responses, and urinary system disorders and abnormalities are some of the common predisposing factors of Candiduria (Nayman Alpat *et al*., 2011).

Although the presence of *Candida* in urine may be a transient and asymptomatic condition and may not meet the typical requirements for systemic antifungal therapy (Bukhary., 2008), the high risk of morbidity

and mortality associated with Candiduria in immunocompromised individuals highlights the clinical importance of this condition (Yang *et al.*, 2008).

Diabetes is the most common endocrine disorder worldwide. Owing to immunologic impairments following diabetes, it is a potent predisposing factor for a wide spectrum of infections. Patients with uncontrolled diabetes and high levels of blood sugar (hyperglycemia) leave the integumentary system, gastrointestinal and urinary tracts, and mucous membranes more susceptible to infectious agents due to reduced immunity of the body (Brieland *et al*., 2001).

Urinary tract infection (UTI) is a frequent condition in diabetic individuals, and Candiduria could be the onset of a disseminated infection (Pallavan *et al*., 2014). According to these data, investigation of the dominant *Candida* species and the relationships between Candiduria and underlying factors seems imperative for effective prevention of severe consequences of disseminated *Candida* infections.

1.3. Objective:

1.3.1. General Objective:

To determine frequency of *Candida* species that associated with urinary tract infection among diabetic patients during March to June 2017 at Advanced Diagnostic Center - Khartoum.

1.3.2. Specific Objective:

1. To isolate and identify *Candida* species from diabetes mellitus patients suffering of UTI.
2. To correlate between the presence *Candida* species in diabetes mellitus that have UTI and different factor (age – gender).
3. To correlate between duration and severity of diabetes mellitus and urinary infection caused by *Candida* species.

Chapter two

Literature Review

2. Literature Review

2.1. Fungal infection

All fungi are eukaryotic organisms, each fungal cell has at least one nucleus and nuclear membrane, endoplasmic reticulum, mitochondria, and secretory apparatus, most fungi are obligate or facultative aerobes. They are chemotrophic, secreting enzymes that degrade a wide variety of organic substrates into soluble nutrients. Nutrients that are then passively absorbed or taken into the cell by active transport (David *et al.*, 2002).

Most pathogenic fungi are exogenous, their natural habitats being water, soil, and organic debris. The mycoses with the highest incidence-candidiasis and dermatophytosis – are caused by fungi that the part of the normal flora. Mycosis may be classified as superficial, cutanenous, subcutaneous, systemic and opportunistic infections have serious underlying disease and compromised host defense (David *et al.*, 2002).

2.2. Diabetes Mellitus

Diabetes mellitus is caused by an absolutes or relative insulin deficiency. It has been defined by the world health organization (WHO), on basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140 mg/dl) or greater than 11.1 mmol/l (200 mg/dl) two hours after the oral ingestion on the equivalent of 75 g of glucose, even if the fasting concentration is normal (Zilva., 1994).

2.3. Classification of Diabetes Mellitus Insulin

2.3.1. Insulin- Dependent Diabetes Mellitus (IDDM, type I)

This term is used to describe the condition in patients for whom insulin therapy is essential because they are prone to develop ketoacidosis. It usually presents during childhood. It has been suggested that many cases

follow a viral infection, which has destroyed the β. Cell of the pancreatic islets (Zilva., 1994).

2.3.2. Non – Insulin – Dependent Diabetes Mellitus (NIDDM, type 2)

Is the commonest variety patients are much less likely to develop ketoacidosis than those with IDDM and although insulin may sometimes be needed, it is not essential for survival. On set is most usual during adult life.Also no genetic markers have been found, there is a familial tendency. A variety of inherited disorders may be responsible for the syndrome, either by reducing insulin secretion, or by causing relative insulin deficiency because of resistance to its action or post receptor defects. (Zilva., 1994).

2.4. Diabetes Mellitus associated with other conditions includes

Absolute insulin deficiency, due to pancreatic disease (Chronic Pancreatitis, hemochromatosis, Cystic Fibrosis).

Relative insulin deficiency due to excessive growth hormone (acromegaly) or increased plasma glucocorticoid concentration due to administration of steroids. Drugs, such as thiazide diuretics (Zilva., 1994).

2.5. Urinary tract infection (UTI):

A urinary tract infection (UTI) is an infection involving the kidneys, ureters, and bladder. These are the structures that urine passes through before being eliminated from the body (Flores *et al*., 2015).

The kidneys are a pair of small organs that lie on either side of the spine at about waist level. They have several important functions in the body, including removing waste and excess water from the blood and eliminating them as urine. These functions make them important in the

regulation of blood pressure. Kidneys are also very sensitive to changes in blood sugar levels and blood pressure and electrolyte balance. Both diabetes and hypertension can cause damage to these organs. Two ureters, narrow tubes about 10 inches long, drain urine from each kidney into the bladder (Flores *et al*., 2015).The bladder is a small saclike organ that collects and stores urine. When the urine reaches a certain level in the bladder, we experience the sensation that we have to void, then the muscle lining the bladder can be voluntarily contracted to expel the urine (Flores *et al*., 2015).

The urethra is a small tube connecting the bladder with the outside of the body. A muscle called the urinary sphincter, located at the junction of the bladder and the urethra, must relax at the same time the bladder contracts to expel urine (Flores *et al*., 2015).The upper urinary tract is composed of the kidneys and ureters. Infection in the upper urinary tract generally affects the kidneys (pyelonephritis), and the lower urinary tract consists of the bladder and the urethra, Infection in the lower urinary tract can affect the urethra (urethritis) or the bladder (cystitis). UTI are much more common in adults than in children, but about 1%-2% of children do get urinary tract infections and are more likely to be serious than those in adults (especially in younger children). Urinary tract infection is second only to respiratory infection as the most common type of infection. These infections are much more common in girls and women than in boys and men younger than 50 years of age. The reason for this is not well understood, but anatomic differences between the genders (a shorter urethra in women) might be partially responsible (Flores *et al*., 2015).

2.6. Urinary Tract Infection (UTI) Symptoms and Signs:

Symptoms of a urinary tract infection (UTI) are similar in men, women, and children. Early symptoms and signs are usually easy to recognize and primarily involve pain, discomfort, or burning when trying to urinate.

Accompanying this can be the sense that one needs to urinate urgently (known as urinary urgency) or the need for frequent urination (called urinary frequency). Even when there is a strong urge to urinate, you may pass only a small amount of urine. The urine itself may appear bloody or cloudy. Men may feel pain in the rectum, while women may experience pain around the pubic bone (Flores *et al.,* 2015).

2.7. Urinary Tract Infections in diabetes mellitus:

Predisposition to urinary tract infections (UTIs) in diabetes mellitus results from several factors. Susceptibility increases with longer duration and greater severity of diabetes. High urine glucose content and defective host immune factors predispose to infection. Hyperglycemia causes neutrophil dysfunction by increasing intracellular calcium levels and interfering with actin and, thus, diapedesis and phagocytosis. Vaginal candidiasis and vascular disease also play a role in recurrent infections (Chen *et al*., 2009).

Recently, the use of SGLT2 inhibitors such as dapagliflozin have led to a small but significant increase in urinary tract infections in patients with inadequately controlled diabetes mellitus. Levels of urinary glucose increased with greater doses of the medication; however, the incidence of urinary tract infections did not. The severity of infections was mild to moderate and responded to the administration of appropriate antibiotics (Johnsson *et al*., 2013).

Over time, patients with diabetes may develop cystopathy, nephropathy, and renal papillary necrosis, complications that predispose them to UTIs Complicated UTIs in patients who have diabetes include renal and perirenal abscess, emphysematous pyelonephritis, emphysematous cystitis, fungal infections, xanthogranulomatous pyelonephritis, and papillary necrosis (Wan *et al.*, 1996).

2.8. Causes of UTI

2.8.1. The bacterial strains that cause UTIs include:

Escherichia coli is responsible for most uncomplicated cystitis cases in women, especially in younger women. *Escherichia coli is* generally a harmless microorganism originating in the intestines. If it spreads to the vaginal opening, it may invade and colonize the bladder, causing an infection. The spread of *Escherichia coli* to the vaginal opening most commonly occurs when women or girls wipe themselves from back to front after urinating, or after sexual activity. *Staphylococcus saprophyticus* accounts for 5 - 15% of UTIs, mostly in younger women. *Klebsiella, Enterococci,* and *Proteus mirabilis* account for most of remaining bacterial organisms that cause UTIs. They are generally found in UTIs in older women, rare bacterial causes of UTIs include *Ureaplasma urealyticum* and *Mycoplasma hominis,* which are generally harmless organisms (Azzarone *et al.*, 2007).

2.8.2. Organisms in severe or complicated infections

The bacteria that cause kidney infections (pyelonephritis) are generally the same bacteria that cause cystitis. There is some evidence, however, *Escherichia coli* strains in pyelonephritis are more virulent (able to spread and cause illness). Complicated UTIs that are related to physical or structural conditions are apt to be caused by a wider range of organism. *Escherichia coli* is still the most common organism, but others include *Klebsiella, Proteus mirabilis,* and *Citrobacter*. Fungal organisms, such as *Candida* species. (*Candida albicans* causes the "yeast infections" that also occur in the mouth, digestive tract, and vagina.). Other bacteria associated with complicated or severe infection include *Pseudomonas aeruginosa, Enterobacter,* and *Serratia* species, Gram-positive organisms

(including *Enterococcus* species), and *S.saprophyticus (*Azzarone *et al*., 2007).

2.9. Urinary tract infections and *Candida albicans*

Anatomically forms urinary tract infections (UTIs) caused by fungi or bacteria– are categorized into two sections (lower and upper tract infections) which may occur in asymptomatic or symptomatic (Behzadi *et al*., 2010).

According to numerous investigations, *Candida* species and in particular, *Candida albicans (C.albicans)* are the most remarkable opportunistic pathogenic fungi causing nosocomial UTIs (Behzadi *et al*., 2015).

Candida albicans and *non–C.albicans Candida* species are considered important parts of microbial normal flora in the oral cavity, alimentary canal and vagina in a vast range of the healthy people. Furthermore, they colonize on the external side of the urethral opening in premenopausal and healthy females. Immune deficiencies may lead to an imbalance between *C.albicans, non–C.albicans Candida* yeasts and the other host normal flora. In this condition, the commensal yeasts of *Candida* may convert into opportunistic pathogenic microorganisms creating *Candidal* UTIs in the host (Behzadi *et al*., 2015). The presence of *C.albicans* and *non–C.albicans Candida* species in urine is known as Candiduria, which may occur in both asymptomatic and symptomatic UTIs (Behzadi *et al*., 2015).

The environmental pH has been shown to influence the rate of germination and elongation of germ tubes. In an acidic medium and in the presence of nitrogenous compounds, it has been observed that *C. albicans* germination is enhanced (Abaitua *et al.,* 1999). A response that may be the result of pH-regulated expression of genes essential to the organism's survival (Abaitua *et al.,* 1999). Such observations would

provide a partial explanation for the greater incidence of *Candida* UTI in such disease states as diabetic ketoacidosis or poorly controlled diabetes conditions that lead to the production of an acidic urine. The enhanced germination may facilitate colonization of the bladder or the urethra and possibly contribute to an ascending infection (Behzadi *et al*., 2015).

The environment that the yeast encounters in the urinary tract will also influence its ability to colonize. *C.albicans* can employ a number of strategies to enable it to survive and evade an immune response. Phenotypic switching can be viewed as a potential powerful virulence factor, because it not only alters the antigenicity of the fungus, but it also affects other factors such as adherence, hydrolytic enzyme production, and germination. Different virulence factors may be used preferentially at different stages of the infectious process. For example, specific SAPs are required for each stage of infection (Staib *et al*., 1999). In addition, the ability of *C. albicans* to alter its repertoire of virulence factors to disseminate and colonize is the key to its success as a pathogen. Far from being a passive opportunist, *C. albicans* can exploit favorable conditions presented by dysfunctional systemic or local defenses to survive or even thrive in the kidney or collecting system. (Behzadi *et al*., 2015).

2.10. Candiduria

Candiduriais categorized into asymptomatic (in healthy people or patients) and symptomatic forms. Symptomatic Candiduria is seen in patients with cystitis, epididymorchitis, prostatis, pyelonephritis and renal candidiasis. However, asymptomatic Candiduria is mostly benign and is not counted as a definite disease. *C.albicans* is one of the most important fungal agents, which may lead to Candiduria (20% of nosocomial infections). A wide range of reported data shows that *C.albicans* ranks first for causing Candiduria among more than 200 *Candida* species (Behzadi *et al*., 2015).

2.10.1. Asymptomatic Candiduria, in previously healthy patient

The presence of Candiduria should be verified with a second, clean-voided urine culture. Many times, it is found that the first culture was contaminated, especially in samples from female patients. Once the presence of Candiduria is confirmed, a careful history and physical examination and screening laboratory studies to look for symptoms or signs of predisposing factors are essential because occult diabetes mellitus, genitourinary structural abnormalities, diminished renal function, and metabolic abnormalities may be discovered (Kauffman., 2002). If no explanation for Candiduria is found, a follow-up examination of the urine is generally all that is necessary because Candiduria can be expected to resolve within weeks to months without therapeutic intervention in the vast majority of individuals (Schonebeck., 1999).

2.10.2.Asymptomatic Candiduria, in predisposed outpatients

The management of outpatients who have Candiduria and who have a predisposing condition is more complicated because yeast in the urine may reflect an invasive infection that requires an antifungal agent for cure. The presence of Candiduria can also be a marker for a process that requires urgent intervention, such as the treatment of a urologic abnormality and associated obstruction. Imaging of the kidneys and collecting system as a baseline in patients with diabetes mellitus or repeating imaging studies in those with known abnormalities of the kidney and collecting system is recommended so that the appropriate treatment can be given.

Antifungal therapy can be avoided in most instances because the long-term consequences of Candiduria are generally benign, even among predisposed patients (Sobel., 1999).

For example, yeast in the urine of an asymptomatic, elderly, diabetic patient with heavy glycosuria may well disappear with tighter control of serum glucose levels. Candiduria that complicates antibiotic therapy frequently resolves shortly after antibiotics are stopped. If benign prostatic hyperplasia and mild obstruction have resulted in asymptomatic Candiduria, a peripherally acting α-adrenergic blocking agent may be all that is required for resolution. Close follow-up of such predisposed patients is prudent (Sobel., 1999).

2.10.3. Asymptomatic Candiduria, predisposed inpatients

In an era of increasing acuity of illness among hospitalized patients, many are predisposed to Candiduria for 1 or more reasons and are unaware of or unable to complain of any associated symptoms. This is especially true if an indwelling bladder catheter is present and the patient is being cared for in an intensive care unit (ICU). Hospitalized patients who have an indwelling bladder catheter are at risk of acquiring yeast in the urine. Platt and colleagues reported that 26.5% of all catheter-associated UTIs (defined as the recovery of >105 organisms per mililiter) were due to *Candida* species (Platt *et al.,* 1986).

The possibility of disseminated candidiasis should be considered in all hospitalized patients with Candiduria, especially in patients in the ICU. Candidemia is common in this setting, and 46%–80% of persons with candidemia will have accompanying Candiduria (Nassoura *et al.,* 1993).

Moreover, *Candida spp* are the fourth most common isolates from blood cultures among hospitalized patients (Wisplinghoff *et al.,* 2004). Despite these observations, candidemia is encountered in <5% of patients in most ICUs (Petri *et al.,* 1997). Thus, most patients with Candiduria probably do not have disseminated infection.

Since many patients in the ICU have an indwelling bladder catheter to monitor urine output, when Candiduria is found, changing or removing

the catheter can be anticipated to clear the Candiduria in 20%–40% of individuals (Sobel *et al.,* 2000). When possible, discontinuing antibiotics that are no longer necessary and treating other predisposing conditions simultaneously should also be done. If Candiduria fails to resolve despite these measures, a more deep-seated infection should be suspected, and imaging of the kidneys and collecting system is indicated. Such studies may reveal renal abscess, fungus ball, or other urologic abnormality accounting for the persistent funguria and that may require an invasive procedure for management.

Antifungal treatment of Candiduria in an inpatient should be reserved for those patients who have solid clinical evidence of infection of the kidney or collecting system or disseminated candidiasis (Kauffman *et al.,* 2000). Until reliable methods for detecting invasive candidiasis in predisposed patients become available, antifungal agents will continue to be used empirically and, many times, inappropriately in this setting. This is unfortunate because for many of these individuals, control or elimination of predisposing factors likely would have resolved Candiduria (Schonebeck., 1972).

2.10.4 Candidal Cystitis and pyelonephritis

The urinary bladder may also be infected by *Candida spp*. Normally, the urinary bladder is sterile, thus, the presence of *Candida spp,* may lead to *Candida cystitis*, which is known as a symptomatic lower UTI. Sometimes, *Candida cystitis* may lead to symptomatic Candiduria. *Candida cystitis* is identified via symptoms of high urination frequency, dysuria and hematuria (Behzadi *et al*., 2015).

Candida pyelonephritis is a severe nosocomial upper UTI which may lead to candidemia and sepsis. The most predominant primary symptoms pertaining to *Candida* pyelonephritis is reported as fever and Candiduria (Behzadi *et al*., 2015).

2.11. Treatment

2.11.1. Antibiotics for treatment of UTI

The usual treatment for both simple and complicated urinary tract infections is antibiotics. The type of antibiotic and duration of treatment depend on the circumstances. Examples of common antibiotics used in treatment include, but are not limited to, amoxicillin, sulfamethoxazole/trimethoprim (Bactrim), ciprofloxacin, nitrofurantoin (Macrobid*)*, and many others (Flores *et al*., 2015).

Lower urinary tract infection (cystitis, or bladder infection). In an otherwise healthy person, a three-day course of antibiotics is usually enough. Some providers prefer a seven-day course of antibiotics. Occasionally, a single dose of an antibiotic is used. A health-care professional will determine which of these options is best. In adult males, if the prostate is also infected (prostatitis), four weeks or more of antibiotic treatment may be required. Adult females with potential for or early involvement of the kidneys, urinary tract abnormalities, or diabetes are usually given a five- to seven-day course of antibiotics. Children with uncomplicated cystitis are usually given a 10-day course of antibiotics. To alleviate burning pain during urination, phenazopyridine (Pyridium) or a similar drug can be used in addition to antibiotics for one to two days (Flores *et al*., 2015). Upper urinary tract infection (pyelonephritis) Young, otherwise healthy patients with symptoms of pyelonephritis can be treated as outpatients. They may receive IV fluids and antibiotics or an injection of antibiotics in the emergency department, followed by 10-14 days of oral antibiotics. They should follow up with their health-care professional in one to two days to monitor improvement. If someone is very ill, dehydrated, or unable to keep anything in his or her stomach because of vomiting, an IV will be inserted into the arm. He or she will

be admitted to the hospital and given fluids and antibiotics through the IV until he/she is well enough to switch to an oral antibiotic. A complicated, acute infection may require treatment for several weeks (Flores *et al*., 2015).

2.11.2. Treatment of *Candida* infection

Today, many types of oral and topical antifungal drugs are commercially available. Depending on candidiasis situations such as Candiduria, cystitis, pyelonephritis, the mycoses therapies differ in antifungal medications (Behzadi *et al*., 2015).

Among a vast range of antifungal drugs, the azole family is the largest one, which inhibits lanesterol 14–β–demethylase activity. The enzyme is involved in the ergosterol biosynthesis pathway and its inactivity may lead to disruption of the fungal cell membrane (Behzadi *et al*., 2015).

In the case of Candiduria, the application of antifungal therapy is absolutely dependedent on microscopic observations and growth cultures. According to the Infectious Diseases Society of America (IDSA) guidelines, asymptomatic Candiduria in patients with no risk factors may be improved either spontaneously or via elimination of indwelling catheters. However, in patients with high risks, the oral use of fluconazole is necessary and unavoidable for preventing invasive candidiasis. Fluconazole is determined as an effective antifungal drug against candiduira caused by *C.albicans* in different age ranges. In the case of asymptomatic Candiduria caused by Fluconazole–Resistant NACA (FRNACA) in patients with high risk, amphotericin B is administered. The antifungal therapy for symptomatic Candiduria may be performed by fluconazole, but, the use of amphotericin B, with or without flucytosine, is recommended for treating symptomatic Candiduria caused by FRNACA species. Either fluconazole or amphotericin B must be used daily for two weeks (Behzadi *et al*., 2015).

2.12. UTI prevention and control

Urinary tract infections can be painful, but can take steps to ease discomfort until antibiotics treat the infection and to reduce risk of urinary tract infections: These tips is:

- Drink plenty of water. Water helps to dilute urine and flush out bacteria.
- Avoid drinks that may irritate bladder. Avoid coffee, alcohol, and soft drinks containing citrus juices or caffeine until infection has cleared. They can irritate bladder and tend to aggravate frequent or urgent need to urinate.
- Use a heating pad. Apply a warm, but not hot, heating pad abdomen to minimize bladder pressure or discomfort.
- Wipe from front to back. Doing so after urinating and after a bowel movement helps prevent bacteria in the anal region from spreading to the vagina and urethra.
- Empty bladder soon after intercourse. Also, drink a full glass of water to help flush bacteria.
- Change birth control method. That contribute to bacterial growth. (Wein., 2016).

2.13. Background studies

Many previous studies were planned to determine the prevalence of uropathogens among diabetes mellitus patients. A study conducted by (Khalifa *et al*., 2009). Those found in their study the prevalence of uropathogens among 135 cases of diabetes mellitus patients was (57%). Most commonly, uropathogens isolated was *Escherichia coli* (23.37%), *Klebsiella* species (23.37%), *Proteus* species (2.59%), *Enterobacter* species (9%), *Pseudomonas* species (7.79%), *Acinetobacter* species

(1.29%), *Staphylococcus aureus* (15.58%), *Staphylococcus saprophyticus* (1.29%) and *Candida albicans (12.98%).*

Other achived study by (Falahati *et al*., 2011) in Iran during April 2015 to September 2015 reported that the prevalence of Candiduria among 305 cases of diabetes mellitus patients was (12.5%). Most commonly isolated was *C.albicans* (31%) and *C.glabrata* (50%).

Chapter Three

MATERIALS AND METHODS

3. MATERIAL AND METHODS

3.1. Study type and design

This was descriptive cross – sectional study.

3.2. Study population

Tow hundreds diabetes mellitus patients (100 of them had symptoms of UTI and another 100 had not).

3.3. Study area and duration

This study was conducted at Center Advanced Diagnostic Center-Khartoum North during March to June 2017.

3.4. Inclusion Criteria

Adult males and females patients who previously diagnosed as diabetics (type 1 or type 2), who were symptomatic or asymptomatic for UTI.

3.5. Exclusion Criteria

To avoid confound factors, patients on antibiotic and antifungal therapy for last 14 days, pregnant women.

3.6. Data Collection method and tool

Data was collected by direct interviewing questionnaire. (Appendix 1)

3.7. Ethical consideration

Permission of the study was obtained from the local authorities in the area of the study and Sudan University of Science and Technology.

Verbal consent was obtained and the objectives of the study were explained to all participants in the study.

3.8. Experimental work

3.8.1. Specimen collection and processing:

Mid stream clean catch urine was collected by each patient in sterile container. Patients instructed how to use the container under aseptic conditions.

3.8.2. Direct Examination of urine

Four ml of urine in centrifuged for 10 minutes at 1500 r.p.m. the supernatant was discharge, and take one drop of deposit in clean slide and cover glass then exanimated in 40X (Cheesbrough, 2006).

3.8.3. Method of Culture

Urine was cultured on sabouraud agar and incubated at 35-37 C° for 24 – 42h, and then colonial morphology was observed (Cream color pasty colonies) (Cheesbrough, 2006).

3.8.4. Gram Stain

Thin smear from growth was prepared and fixed. The fixed smear was covered with crystal violet stain for 30 – 60 second. The stain was washed off rapidly with clean water. The smear was covered with lugol's iodine for 30 – 60 second, and washed off with clean water and decolorized rapidly (few seconds) with acetone – alcohol, then washed immediately with clean water. The smear was covered with neutral red stain for two minutes, washed, air dried and examined with 100 X objective (Cheesbrough, 2006).

Candida species appeared as Gram-positive yeast.

3.8.5. Identification of *Candida* species

3.8.5 1.Germ tube test

Human serum (0.5 ml) was pipetted into a small test tube and by sterile wires loop the serum was inoculated with a yeast colony. The tube was placed in water bath in 35 – 37 C° for 2 – 3 hours, then using Pasteur pipette, a drop of the serum yeast culture was transferred to glass slid and was covered with a cover glass.

The preparation was examined using the 10 X and 40 X objectives. Germ tube appeared as hyphal –like extensions of yeast cells that produced without constriction at the point of origin (Cheesbrough, 2006).

3.8.5.2. Chrom Agar *Candida*

Plates of chrom Agar was allowed to room temperature to be warmed before using .The yeast colony was inoculated and streaked using a sterile loop. The plates were incubated in an inverted position, protected from the light, aerobically at 35ºC for 24-48 hours.

Examined colors of colony was determine the type of *Candida* species. *Candida albicans* was appeared as high green color; *Candida glabrata* was appeared as light pink (Haley., 1980).

3.8.5.3. Corn meal Agar

Corn meal agar morphology was used as a mean to determine whether the yeast produce blastoconidia. Corn meal agar can be used to detect characteristic chlamydoconidia produced by *C.albicans*. (Patricia., 2014).An inoculated needle was used to obtain visible paste of the organism. The needle was drawn through the agar to make two perpendicular lines in the shape of an "x". Coverslip was Flamed and allowed to cool. Once coverslip was cooled, it will be placed over the central area of the "x" in order to reduce the oxygen tension. Reduced oxygen tension stimulates chlamydospores production. The plate was sealed with tape and incubated aerobically at room temperature (25-30ºC) for up to 72 hours in the dark. Typical colonial growth and morphology was examined daily. The plate was inverted and examined microscopically using a low power objective (10X). Chlamydospores formation was view along the edge of the coverslip (Campbell., 1980).

3.9. Data analysis

Collected data was analyzed using the application Statistical Package of Social Science (SPSS) version 16.0. Example independent sample test and chi-square test.

Chapter Four

Results

4. Results

A total of 200 diabetes patients who attending Advanced Diagnostic Center in Khartoum North, were enrolled in this study to detect frequency of *Candida* species associated with UTI, during period from March to June 2017.

Out of the total 5 (2.5%) patients were positive for *Candida* infection while the remain 195 (97.5 %) were negative, demonstrated in table (1).

The overall results demonstrated that their frequency of *Candida* species was 4 (80 %) and 1(20%) among symptomatic and asymptomatic diabetic patients respectively (Table 1), this different in the isolation was not statistically significant (P=0.17).

Four from this five *Candida* infection were due to *C. albicans* infection and other one is due to *C. glabrata*.

Regarding to age most of *Candida* UTIs was observed among 41-60 age range in symptomatic group and asymptomatic group which is demonstrated in table (2).

According duration of diabetes mellitus in symptomatic group most of *Candida* UTIs was observed in 1-10 years duration and 11-20 years in asymptomatic group which is demonstrated in table (3).

Referring to gender most of *Candida* UTIs was observed among symptomatic females and asymptomatic males. Which is demonstrated in table (4).

The statistical correlation of *Candida* infection for age is (P=0.63), for gender is (P=0.35), and for the duration of disease is (P=0.47) .The results showed insignificant relation (P>0.05).

Table (1): Frequency of *Candida* species UTI infection among symptomatic and a symptomatic patient

Results / Diabetic patients study groups	Positive for *Candida* Species	Negative for *Candida* species	Total
UTI symptomatic patients	4(4%)	96(96%)	100(100%)
UTI asymptomatic patients	1(1%)	99(99%)	100(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

p.value= 0.17

Table (2): Frequency of *Candida* species UTI among study population regarding their age distribution

Age in years	Symptomatic diabetic patient		Asymptomatic diabetic patient		Total
	Positive	Negative	Positive	Negative	
21 – 40	1(25%)	19(19.80%)	0(0%)	21(21.22%)	41(20.5%)
41 – 60	2(50%)	54(56.25%)	1(100%)	54(54.54%)	111(55.5%)
61 – 80	1(25%)	23(23.95%)	0(0%)	24(24.24%)	48(24%)
Total	4(100%)	96(100%)	1(100%)	99(100%)	200(100%)

p.value= 0.63

Table (3): Frequency of *Candida* species UTI among study population regarding their diabetes mellitus duration distribution

Duration in years	Symptomatic		Asymptomatic		Total
	Positive	Negative	Positive	Negative	
1 – 10	4(100)%	70(72.92%)	0(0%)	73(73.7%)	147(73.5%)
11 – 20	0(0%)	20(20.84%)	1(100%)	19(19.1%)	40(%20)
21 – 30	0(0%)	5(5.20%)	0(0%)	6(6.1%)	11(%5.5)
31 – 40	0(0%)	1(1.04%)	0(0%)	1(1.10%)	2(%1)
Total	4(100%)	96(100%)	1(100%)	99(100%)	200(100%)

p.value= 0.47

Table (4): Frequency of *Candida* species UTI among study population regarding their gender distribution

Gender	Symptomatic		Asymptomatic		Total
	Positive	Negative	Positive	Negative	
Males	1(25%)	41(42.70%)	1(100%)	45(45.46%)	88(44%)
Females	3(75%)	55(57.30%)	0(0%)	54(54.54%)	112(56%)
Total	4(100%)	96(100%)	1(100%)	99(100%)	200(100%)

p.value= 0.35

Chapter Five

Discussion, conclusion and Recommendation

5. Discussion, Conclusion and Recommendations

5.1. Discussion

Diabetes mellitus is often simply considered as diabetes, a syndrome of disordered metabolism with abnormally high blood glucose levels (hyperglycemia). The two most common forms of diabetes are type 1 diabetes (diminished production of insulin) and type 2 diabetes (impaired response to insulin and β-cell dysfunction). Both lead to hyperglycemia, excessive urine production, compensatory thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism (Yi and Zhongjie., 2010).

Fungal urinary tract infections due to *Candida* has increased significantly in the last years. Diabetes mellitus is one of the predisposing factors to fungal urinary tract infections, *Candida albicans* is the most common isolated species, but non *Candida albicans* also cause fungal UTIs, and in many centers worldwide, non *Candida albicans* predominate (Arjun .,2017).

This study was conducted to detect the frequency of *Candida* species associated with UTI among diabetes mellitus patients.

In this study, the results showed that the frequency of *Candida* species infection among symptomatic diabetes mellitus patients was 4%, and among asymptomatic diabetes mellitus patients respectively was 1%. The statistical analysis of results showed that there is no correlation between cadiduria infections among diabetes mellitus patients of age, gender and duration of disease. Which is similar to another study was made in Universidad Nacional Autónoma de México (UNAM) by (González *et al.,* 2006),which showed prevalence of *Candida* species infection among diabetes mellitus patients was (5.1%), and there was no correlation

between cadiduria infections among diabetes mellitus patients of age, gender and duration of disease.

However, another study done in Gondar University Hospital among Ethiopian patients by (Yismaw *et al*., 2013). Result obtained by their study in 100 symptomatic patients 17.1% were positive and another 100 asymptomatic patients 7.5% were positive. Difference in results may be due to difference in environment and behaviors of sudanese and ethiopian patients.

In our research, the detection of *Candida* species associated with UTI used by conventional method. However, a similar study in Sudan made by (Mohamed Elsheikh., 2009) used molecular technique instead of conventional method and found that the prevalence of different fungi associated with UTI among diabetes mellitus patients was about 23%

5.2. CONCLUSION

This research concluded that there was low frequency of *Candida* species infection among diabetes mellitus patients and statistically there were insignificants relations between *Cadiduria* among diabetes mellitus patients with age, gender and duration of disease

5.3. RECOMMENDATIONS

Molecular techniques may be used routinely with culture to avoid false negative result and sample size should be increased in subsequent relatives researches.

REFERENCES

6. REFERENCES

1. Abaitua, F; Rementeria,A; and San Millan,R; *et al*. (1999). In vitro survival and germination of *Candida albicans* in the presence of nitrogen compound. *Microbial*. **145(6):**1641-7.

2. Apurba, S.S; and Sandhya, B.K. (2016). Essentials of medical microbiology. Jaypee Brothers Medical Publishers (LTD). New Delhi. Page 566.

3. Arjun; B, Ashim; K, H, and Pijush; K, Datta. (2017). Spectrum of Cutaneous Manifestations of Diabetes Mellitus: An Observational Study from a Tertiary Care Hospital. *J Diabetes Metab.* **9(4):**791.

4. Azzarone, G; Liewehr, S, and O'Connor, K. (2007). Cystitis. *Pediatr Rev.* **28(12):**474-6.

5. Behzadi, P; Behzadi, E; Yazdanbod, H; Aghapour, R; Cheshmeh, M.A, and Omran, D. **(2010).** Urinary tract infections associated with *Candida albicans*. *Maedica*. **5(1):**277–279.

6. Behzadi, P; Behzadi, E; and Ranjbar, R. (2015*).* Urinary tract infections and *Candida albicans. Maedica*. **68(1):**96-101.

7. Bonadio, M; Costarelli, S; Morelli, G; and Tartaglia, T. (2006). The influence of diabetes mellitus on the spectrum of uropathogens and the antimicrobial resistance in elderly adult patients with urinary tract infection. *Current Medical Mycology*. **2423-3439(3):** 10–14.

8. Brieland, J; Essig, D; Jackson, C; Frank, D; Loebenberg, D; and Menzel, F; et al. (2001). Comparison of pathogenesis and host immune responses to *Candida glabrata* and *Candida albicans* in systemically infected immunocompetent mice. *Infect Immun*. **69(8):**5046–55.

9. Bukhary, Z.A. (2008). Candiduria: a review of clinical significance and management. *Saudi J Kidney Dis Transpl*. **19(3):**350–60.

10.Campbell; M, C. (1980). Corn meal agar with tween® 80. HardyDiagnostics.1996.

11. Chen SL, Jackson SL, Boyko EJ. (2009). Diabetes mellitus and urinary tract infection: epidemiology, pathogenesis and proposed studies in animal models. *J Urol*. **182(6 Suppl):** 51-6.

12. Chessbrought, M. (2006). District laboratory practice in Tropical countries. Cambridge university press. New York. Page 108-112.

13. David, G; Richard, C.B; and John, F.P. (2002). Medical microbiology. Library of congress. London. Page 623.

14. Falahati, M; Farahyar, S; Akhlaghi, L; Mahmoudi, S; Sabzian, K; Yarahmadi, M; and Aslani, R. (2016*).* Characterization and identification of Candiduria due to *Candida* species in diabetic patients. *Current Medical Mycology*. **2(3):**10-14.

15. Fisher, J , F; Chew, W,H; Shadomy, S; Duma, R,J; Mayhall, C,G; and House, W,C. (1982). Urinary tract infections due to *Candida albicans. PubMed*. **4(6):**1107-18

16. Flores, M; Ana, L; Jennifer, N; Walker, M. C, and Scott, J. H. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol*. **13(5):** 269-284

17. Foxman, B. (2002). Epidemiology of urinary tract infections: incidence, morbidity and economic costs. *Am J Med*. **113(1):5**–13.

18. Fünfstück, R; Nicolle, L.E; Hanefeld, M; and Naber, K.G. (2012). Urinary tract infection in patients with diabetes mellitus. *Clin Nephrol*. **77(1):**40–48.

19. González, P; Avilés, A; Luís, H; R; Luna, A; J; Dávila, M;R and Ortiz, Zaragoza;C. . (2006). Urinary tract infection by *Candida* species. *PubMed*. 38(3):147-53.

20. Gougeon; R, Meshell; C, and Catherine J. Field. (2006). The impact of low-carbohydrate diets on glycemic control and weight

management in patients with Type 2 diabetes. *Canadian Journal of Diabetes* .**30(3):** 269.

21. Haley; L.D. (1980). Criterion™ Sabouraud Dextrose (SABDEX) agar. HardyDiagnostics. 1996.

22. Johnsson, K.M; Ptaszynska, A; Schmitz, B; Sugg, J; Parikh, S.J, and List J, F. (2013). Urinary tract infections in patients with diabetes treated with dapagliflozin .*J Diabetes Complications*.**27 (5):**473-8.

23. Kauffman, C.A; Vasquez, J.A, and Sobel, J.D. (2000). Prospective multicenter surveillance study of funguria in hospitalized patients. *The National Institute for Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis*. **6**: **(30**)14-8.

24. Kauffman, C.A. (2002). Candiduria: diagnostic and treatment conundrums, Curr Treat Opinions Infect Dis. *Clinical Infectious Diseases*. **6(52):** 457–466.

25. Khalifa, S. G; Einass, E; Nuri, B; Rania, A.N; Salwa, F. A; Amal,R; Nadia,S; Mohamed, A,E; Taher,B ; and Johan, D.K. (2009). Uropathogens from diabetic patients in Libya: virulence factors and phylogenetic group of Escherichia coli isolates. *J. Med. Microb **58***, 1006-1014.

26. Mohammed. A; Elsheikh. (2009). prevalence of fungal infection in Sudanese Diabetic patient in Khartoum state. M.Sc. thesis Sudan University of Science and Technology.

27.Nassoura, Z; Ivatury,R.R;Simon,R.J; Jabbou, M, and Stahl,W.M. (1993). Candiduria as an early marker of disseminated infection in critically ill surgical patients: the role of fluconazole therapy. *J Trauma*. **(6) 35:** 290-5.

28.NaymanAlpat, S; Özguneş,I ; Ertem, O.T; Erben, N; DoyukKartal, E; and Tözun, M. et al. (2011). Evaluation of risk factors in patients with Candiduria. *Mikrobiyol Bul*. **45(2):** 318–24.

29. Pallavan, B; Ramesh, V; Dhoanasekaran, B.P; Oza, N; and Govindarajan, V. (2014). Comparison and correlation of *Candida*l colonization in diabetic patients and normal individuals. *Curr.Med.Myc.* **2423-3439:(3)**10–14.

30. Patricia M, T, (2005). Diagnostic microbiology. South Dakota state university. South Dakota. Page 779.

31. Petri, M.G; Konig, J, and Moecke, H.P. (1997). Epidemiology of invasive mycosis in ICU patients: a prospective multicenter study in 435 non-neutropenic patients. Paul-Ehrlich Society for Chemotherapy, Divisions of Mycology and Pneumonia Research, Intensive Care Med. *Clin. Infect. Dis.* **6(52):** 457–466.

32. Platt, R; Polk, B.F; Murdock, B, and Rosner, B. (1986). Risk factors for nosocomial urinary tract infection. *Clin. Infect. Dis.* **6(52):** 457–466.

33. Rother, K.I. (2007). Diabetes Treatment-Bridging The Divide. Nengl J Med **356(15):**1499-150.

34. Schappert, S.M. (1999). Ambulatory care visits to physician offices, hospital outpatient departments, and e.mergency departments. United States, 1997. *National Center for Health Statistics. Vital Health Stat.***13 (143):**1–39.

35. Schonebeck, J. (1937). Studies on *Candida* infection of the urinary tract and on the antimycotic drug 5-fluorocytosine. *Cli.Infect.Dis.* **6(52):** 457–466.

36. Shah, B.R; and Hux, J.E. (2003). Quantifying the risk of infectious diseases for people with diabetes. *Diabetes Care*. **26(2**):510–513.

37. Sobel, J, D. (1999). Management of asymptomatic Candiduria. Clinical Infectious Diseases. **6(52):** 457–466.

38. Sobel, J. D; Kauffman, C.A, and McKinsey, D. (2000). Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. *Clinical Infectious Diseases*, **6(52):** 457–466.

39. Staib, P; Kretschmar, M; and Nichterlein, T; et al. (1999). Host-induced, stage specific virulence gene activation in *Candida albicans* during infection. *Mol Microbiol* .**139(32):**533-46.

40.Teodora, C; Bogdan, T; Delia, M; Luminiţa, B; Florin, H; Elena, H; Roxana, M; Romulus, T; and Monica, L. (2017). Urinary tract infections in Romanian patients with diabetes: prevalence, etiology, and risk factors. *Current Medical Mycology*. **2423-3439(14):** 1-7.

41. Wan, Y,L; Lee, T,Y; Bullard, M,J; and Tsai, C,C. (1996). Acute gas-producing bacterial renal infection: correlation between imaging findings and clinical outcome. *Radiology*. **198(2):**433-8.

42. Wein; A.J. (2016). Urinary tract infection (UTI). Mayo Clinic Staff. Aug. 25, 2017. https://www.mayoclinic.org/diseases-conditions/urinary-tract-infection/diagnosis-treatment/drc-20353453.

43. William, A ; Strohl , Pamela, C; Richard, A; Pamela, C, and Harriet Rouse. (2003). Essentials of Clinical Mycology. New York. Oxford University. Page 265-266.

44.Wisplinghoff H, Bischoff T ,Tallent SM, Seifert H, Wenzel RP, Edmon MB. (2004). Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study, *Clin Infect Dis*. **6(39):**309-17.

45. Yang, Y.L; Cheng, M.F; Chang, Y.W; Young, T.G; Chi, H; and Lee, S.C; et al. (2008). Host factors do not influence the colonization or infection by fluconazole resistant *Candida* species in hospitalized patients. *Current Medical Mycology*. **2423-3439:(3)**10–14.

46. Yashavanth, R; Shiju, M; Bhaskar, U; Ronald, R; and Anita, K. (2013). Candiduria: prevalence and trends in antifungal susceptibility in a tertiary care hospital of mangalore. *J ClinDiagn Res*.**7 (11):**2459–61.

47. Yilin and Zhongjie Sun. (2010). Current views on type 2 diabetes. J Endocrinol. **204(1):** 1.

48. Yismaw, G; Asrat, D; Woldeamanuel, Y;and Unakal, C. (2013). Prevalence of Candiduria in diabetic patients attending Gondar University Hospital, Gondar, Ethiopia. *pubmed.* **7(2):**102-7.

49. Zakeya, A, B. (2008). Candiduria: A Review of Clinical Significance and Management. *Saudi journal of kidney diseases and transplantation*. **3(19):**350-360.

50. Zilva, P.M. (1994). Clinical chemistry in diagnosis and treatment. British library cataloguing. London.**182 (6 Suppl):** 51-6.

.

Appendices

Appendix 1

Questionnaire

Date:

a) General information

-Serial No….....

-Age……

-Gender…..... male ( ) female ( )

b) Clinical information

1- Symptom of UTI:-

- Urinary frequency Yes ( ) No ( )
- Dysuria Yes ( ) No ( )
- Hematuria Yes ( ) No ( )
- Supra pubic pain Yes ( ) No ( )
- Pyrexia Yes ( ) No ( )
- Lion pain Yes ( ) No ( )

2- on antibiotic

If yes specify……

3-Duration of DM……

4- Family history of UTI Yes ( ) No ( )

5- Previous UTI Yes ( ) No ( )

If yes, when…….

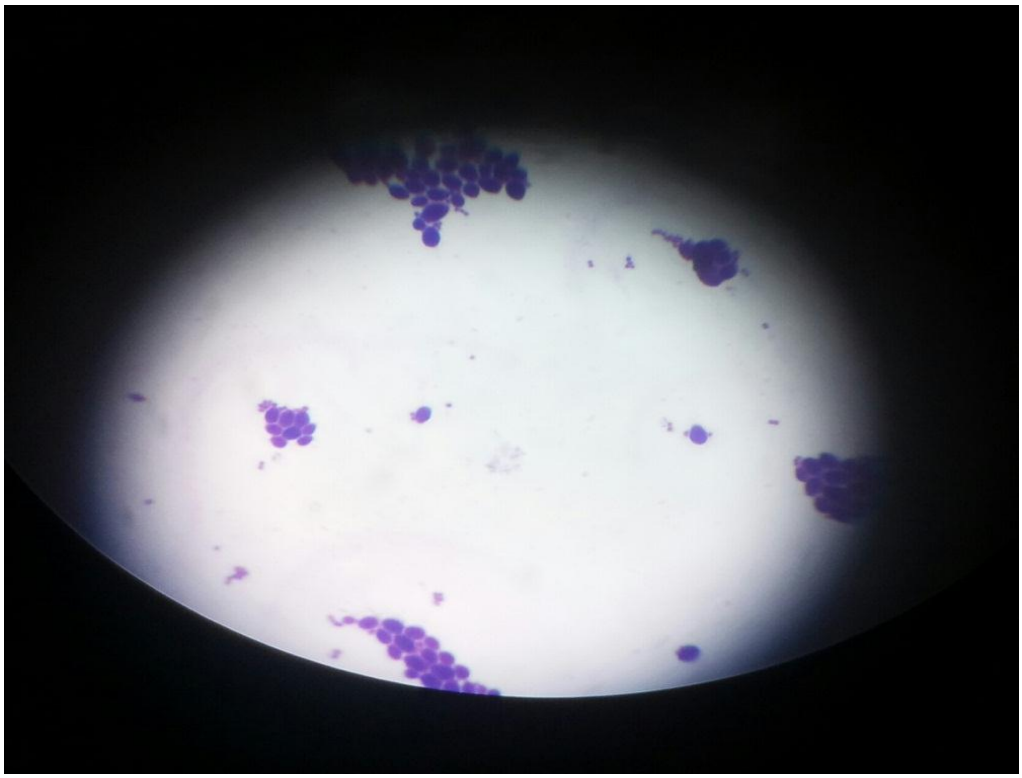
Specimen: urine ( ) other ( )

Cultuer result…….

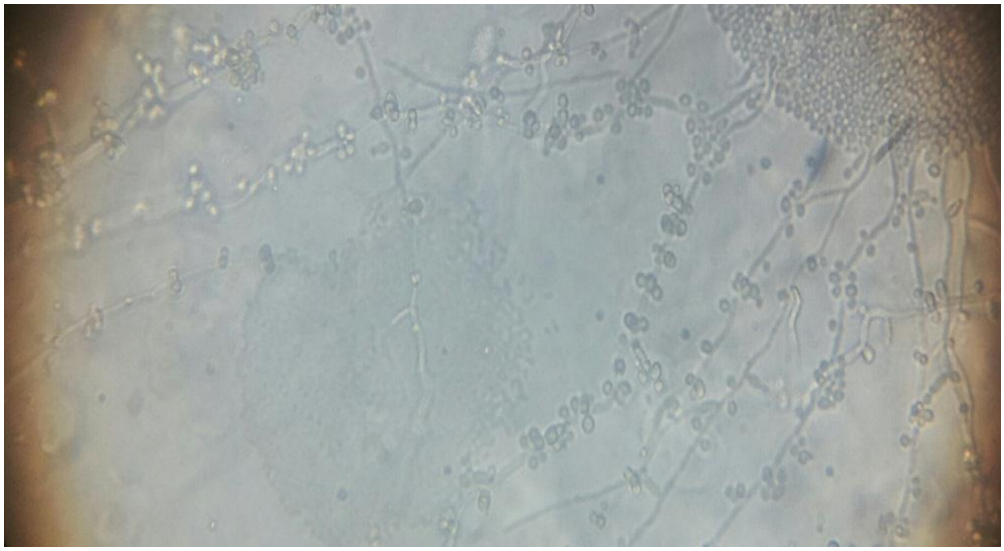
Appendix 2

Image

Candida species culture in Cystine Lactose Electrolyte Deficient Agar

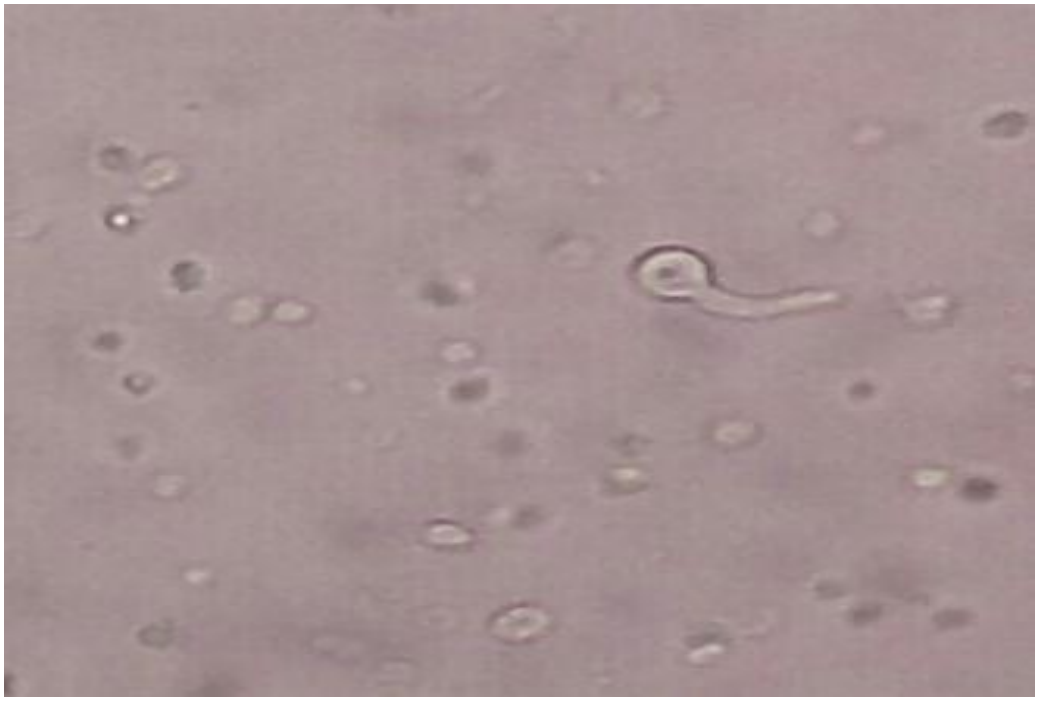


***Candida* species in Gram stain, it Gram positive yeast**



***Candida albicans* in cornmeal agar**

***Candida* species in chromagar**



Germ tube test for *Candida albicans*