UJMR, Vol. 9 No. 1, June, 2024, pp. 109 - 115 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2491.012

Received: 13 September 2023 **Accepted:** 06 May 2024

The Study of *Candida albicans* among Diabetic Patients Attending Some Selected Hospitals in Sokoto Metropolis

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Abstract

Candidiasis is an infection caused by the yeast genus Candida that lives in or on certain parts of the body, such as the gastrointestinal tract, the respiratory tract, the vagina, and even the skin. This study aimed to determine the prevalence of Candida albicans among diabetic patients attending some selected hospitals in Sokoto metropolis. A total of 245 early-morning midstream urine samples were inoculated on SDA Saboraud dextrose agar. The isolates were identified using Gram staining, germ tube test, and KOH test. The prevalence of Candidiasis was 9.4%. Females had a higher prevalence of 5.7% compared to males (3.7%) regarding gender (P = 0.247). Patients aged 30-34 years, 35-49 years, 40-44 years, 45-49 years and 50-54 years had prevalence of 0.0%, 0.4%, 0.4%, 0.4% and 8.0% respectively. Patients aged 55 years and above were the most infected in this study (7.3%) (P = 0.796). Due to high blood glucose levels and old age, regular clinic check-ups are highly recommended to prevent avoidable complications. These findings suggest that women are the most vulnerable compared to men. Therefore, it is necessary to continue evaluating the Candida albicans infection rate in diabetic patients in hospitals and the community.

Keywords: Candida albicans, prevalence, hospital, patience, Sokoto.

INTRODUCTION

Candidiasis is a disease caused by the yeast genus *Candida*. *Candida* is associated with diabetes patients since it is a yeast that adores and flourishes on sugar (Sule *et al.*, 2020).

Candida albicans have a two-layered cell wall comprising a core of the ß-glucanosylchitin skeleton responsible for the cell wall's strength and shape. Chitins are found in the inner part of the cell wall, and their chains can form tight, antiparallel, hydrogen-bonded structures associated with a high level of insolubility. *Candida* has one CHS (Chitin synthase) family of four genes, one of which is class II CHS1. This CHS1 forms septums, cell viability, shape, and integrity (Garcia-Rubio *et al.*, 2020).

Fungal diseases are one of the leading causes of death and illness worldwide, affecting more than 1.5 billion people each year (Bongomin *et al.*, 2017). In Nigeria, the number of people affected by fungal diseases is estimated to be 11.8%, with *Candida albicans* being the most common and invasive pathogen in hospitalized patients (Bashir *et al.*, 2022). This fungal species is highly adaptable and has a wide range of virulence factors, allowing it to transform from a commensal organism into a pathogen. One of its key virulence features is its ability to change morphologies from pseudohypha to hypha, yeast to pseudohypha, and vice versa. The main distinction between the yeast and hyphal forms is the hyphal wall's chitin content, which is slightly higher than in the yeast form. Cell wall mannans also differ between the morphotypes, with the hyphal form having a significantly lower number of phosphodiesterified acids-labile, acid-stable, and acid-stable side chains. The cell wall of *Candida albicans* is made up of two layers. The primary layer comprises a skeleton comprising glucose and glucose-chitin (Garcia-Rubio *et al.*, 2020).

Diabetes mellitus is a long-term condition in which the body cannot regulate its blood sugar levels, making the body more susceptible to other diseases, such as Candidiasis. *Candida* species are opportunistic organisms and are usually not pathogenic, except in an immune-compromised host, where they can cause diseases ranging from superficial in appearance (the outer layer of the skin's stratum corneum) to disseminated infection (infections of the lungs, vagina, and other body parts) (Sule *et al.*, 2020).

Diabetic patients develop resistance and recurrent infections, apart from slow healing from wounds or injuries. It is generally thought that the condition is caused by the malfunctioning of leukocytes, particularly in patients with uncontrolled blood glucose. However, *Candida* disease is more concerning, especially in patients with severe hyperglycemia (Sule *et al.*, 2020).

The main factors that contribute to the development of Candidiasis are the formation of biofilms, the production of acidic proteinases, and phospholipases. Once the yeast makes contact with the host, it produces enzymes that break down or damage the cell membrane or extracellular protein, allowing it to enter into the cell, while phenotypic switch or platelet coating may help it avoid the immune system. Many studies have indicated that the rate of yeast infection in patients with diabetes could be as high as 54% and that most of the isolates could be 25% to 69% of *candida albicans*. The genus *Candida* consists of 150-200 species. *Candida* used to be the only species that caused infection in patients with diabetes, with other species, such as *Candida parapsilosis* and *Candida tropicalis*, occasionally causing infection (Chouhan *et al.*, 2019).

Candidiasis was uncommon in the past and was largely overlooked. However, with the introduction of invasive devices, immune suppression therapy, and broad-spectrum antimicrobial drugs over the long term, Candidiasis has become a major health challenge. People with diabetes are particularly vulnerable to Candidiasis, as their blood glucose levels are high (Bashir *et al.*, 2022).

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted among diabetic patients attending a selected hospital in Sokoto metropolis, Sokoto state, Nigeria.

$$n = \frac{Z^2 P \times q}{d^2}$$ (Thrustfield, 1997)

Where Z= standard deviation at 95% confidence interval=1.96
P=prevalence value = 20% (Karwiti *et al.*, 2022)
q= 1-P=1-0.2=0.8
d=degree of confidence=5%=0.05

$$n = \frac{(1.96)^2 \times 0.2 \times 0.8}{(0.05)^2}$$

$$n = \frac{3.842 \times 0.2 \times 0.8}{0.0025}$$

n=245

Ethical approval

Ethical approval for the study was obtained from the research and ethics committee of the Ministry of Health and Specialist Hospital Sokoto, respectively (Ref: No. SMH/1580/V. IV).

Study population

The study populations were diabetic patients of all age groups attending selected hospitals in Sokoto metropolis.

Study area

The study was conducted at Sokoto Specialist Hospital and Maryam Abacha Women's and Children's Clinic, Nigeria. The Specialist Hospital is on Sultan Abubakar Road, Sokoto, while the Maryam Abacha Women and Children Clinic is on Sultan Bello Road, Sokoto. Sokoto is located at longitude 5° 15' 00 East and latitude 13° 05' 00" North in degrees, minutes, and seconds (Collins Maps, 2018). It shares borders with the Republic of Niger to the north, Kebbi State to the west and southwest, and Zamfara State to the east. This region experiences two seasons: the dry season (October-April and lasting until May or June) and the rainy season (May-September or October). The agents that cause Candida are considered present at any time of the year, regardless of season and other climatic conditions such as temperature and relative humidity. The main occupation of the people is farming and animal husbandry. Sokoto State has a population of 3,696,999 according to the 2006 census, with an area of 28,232.37 km2, and is largely comprised of Hausa and Fulani and others. Sociocultural characteristics are homogenous, as the majority of Indigenous people are Muslims (UNFPA, 2012).

Inclusion criteria

Male and female patients of all age groups were considered for this study. Both outpatients and inpatients diagnosed with diabetic mellitus were included.

Exclusion criteria

Non-diabetic patients were excluded in this study. Diabetic patients who are on antifungal chemotherapy were also excluded.

Sample size

The sample size was calculated using the following the following formula;

Questionnaire administration

Questionnaires were administered to each of the consented participants. The parameters used are sex, age, tribe, education, religion, occupation, marital status, history of diabetic mellitus, and history of antibiotics used.

Sample collection and processing

Each diabetic patient was asked to collect a clean midstream urine sample.

Urine samples of 10 ml to 15 ml were collected in sterile wide-mouth screw-cap containers from each diabetic patient. The vial was labeled with a unique sample number and the date and time of collection and was immediately transported to the Medical Microbiology Laboratory in the School of Medical Laboratory Sciences for analysis (Yismaw *et al.*, 2013).

Culture of samples

In the laboratory, samples were inoculated onto prepared Saboraud Dextrose Agar (SDA) plates supplemented with chloramphenicol (16 mg/ml), then incubated at 30°C for 72 hours.

Microscopic techniques

Direct wet preparation

Urine samples were placed on a grease-free glass slide with a coverslip and examined under a microscope using an objective lens (×10 and ×40) to detect the presence of pus cells, red blood cells, casts, crystals, and yeast cells.

Direct KOH mount

Urine samples were tested directly with a 10% KOH smear. This is necessary to differentiate between yeast and bacterial growth. The wet preparation examination also revealed the size, shape, number of buds, and the type of attachment to the yeast cells. The sample was emulsified in a drop of KOH on a clean glass slide, covered with a coverslip, and examined under a microscope using ×10 and ×40 objective lenses for budding cells (Ochei and Kolhatkar, 2010).

Gram staining technique

Smear of urine samples were made on clean, grease-free glass slides. Heat fixed the smear by passing it over the flame 3 to 4 times. The smear was soaked with crystal violet for 60 seconds, rinsed with clean water, then soaked with Lugol's iodine for 30 seconds. The smear was decolourized with alcohol acetone, immediately washed with water, and restained with Safranin for 30 seconds. The stain was washed with clean water and allowed to air dry, then examined under a microscope at ×100 magnification using immersion oil (Ochei and Kolhatkar, 2010).

Isolation and identification

After two days, the colonial morphology of the fungal growth was observed macroscopically. The colony's color, shape, and texture were examined to identify the yeast. Colonies that were white or cream-colored were subjected to germ tube tests. Germ tube positive indicated *Candida albicans* (Ochei and Kolhatkar, 2010).

Germ tube test

About 500 µl (0.5 ml) of human serum was pipetted into a small test tube. Using a sterile wire loop, the serum was inoculated with yeast colonies from the culture plate, and then the tube was incubated in an incubator at 37°C for 3 hours. Using a Pasteur pipette, a yeast culture serum was transferred to a glass slide and covered with a coverslip; the preparation was examined under a microscope using x10 and x40 objectives with condensed iris diaphragms. The condenser is closed enough to create good contrast (Cheesbrough, 2006). The appearance of small, sprouting tube-like outgrowths or filaments projecting from the cell surface confirms the production of the germ tube.

Statistical analysis

The data obtained were analyzed using SPSS (version 20). The proportion of *Candida albicans* isolated from urine samples and their prevalence were compared using the Pearson chi-square test. P value < 0.05 was considered statistically significant.

RESULTS

Of the 245 participants recruited into the study, 52(21.3%) were Gram-positive, of which 9.4% were infected with *Candida albicans*, as confirmed by the germ tube test (Table 1). Women had a 5.7% infection rate, while men had a 3.7% rate (Table 2).

The blood sugar levels of *Candida albicans*-positive subjects were compared with those negative for *Candida albicans*. It can be seen that the average blood sugar level of people positive for *Candida albicans* was higher than that of people negative for *Candida albicans*. The p-value obtained from the test showed a statistically significant difference between the two blood sugar levels ($P \leq 0.002$) (Table 3).

Table 1: Prevalence of *Candida albicans* among Diabetic Patients in Sokoto State.

Isolate	Results	Frequency	Percentage
Candida albicans	**Positive**	23	9.4%
	Negative	222	90.6%
	Total	245	100%

Note: X^2 = Chi-square

Table 2: Prevalence of *Candida albicans* among Diabetic Patients in Sokoto State based on Gender.

Gender	Results	Frequency	Percentage	χ^2	P-value
Male	**Positive**	9	3.7%	1.339	0.247
	Negative	115	46.9%		
Female	**Positive**	14	5.7%		
	Negative	107	43.7%		
	Total	245	100		

Note: X^2 = Chi square

Table 3: Assessment and Comparison of Blood Sugar Level between Diabetic Patients (Positive and Negative) Tested on Culture

Variables	No of Samples	Mean ±SD	F	P-value
Positive	52	9.579±2.516	9.83	0.002
Negative	193	8.283±2.677		

x^2= Chi-square

F = ANOVA

Of the total 52 positive samples (21.2%), only 32 samples (13.1%) were Gram-positive, while 20 samples (8.2%) were Gram-negative. It was also revealed that 23 (9.4%) germ tube tests were positive, and 29 (11.8%) were negative.

Research subjects aged 55 years and older were the most infected in the study area (7.3%), while those under 35 had a very low risk of *Candida albicans* infection (0.0%). The participants' age difference was not statistically significant (p = < 0.05). (Table 4).

The Hausa ethnic group was the most infected in the study area, with a *Candida albicans* infection rate 19 (7.8%). The p-value shows that statistically, there were no significant differences between subjects in terms of ethnicity (Table 5).

It was observed that housewives had the highest prevalence of 9(3.7%) compared to civil servants (2.1%), while businessmen had 6 (2.4%). The difference in occupation between participants in this study was not statistically significant (p = 0.05) (Table 6)

Table 4: Prevalence of *Candida albicans* based on Age Groups.

Age Groups (years)	Germ Tube test		χ^2	P-value
	Positive	Negative		
30-34	0(0.0%)	1(0.4%)	2.366	0.796
35-39	1(0.4%)	1(0.4%)		
40-44	1(0.4%)	1(0.4%)		
45-49	1(0.4%)	4(1.6%)		
50-54	2(0.8%)	3(1. 2%)		
≥ 55	18(7.3%)	19(7.8%)		
Total	23(9.4%)	29(11.8%)		

Table 5: Prevalence of *Candida albicans* based on Ethnicity

Ethnicity	Germ Tube test		χ^2	P-value
	Positive	Negative		
Hausa	19(7.8%)	20(8.2%)	1.273	0.259
Fulani	4(1.6%)	9(3.6%)		
Total	23(9.4%)	29(11.8%)		

Note: X^2 = for Chi-square

Table 6: Association of *Candida albicans* Infection and Occupation among Diabetic Patients in Sokoto

Occupation	Germ Tube test		χ^2	P-value
	Positive	Negative		
House Wife	9(3.7%)	11(4.4%)	0.062	0.996
Civil Servant	5(2.1%)	7(2.9%)		
Business	6(2.4%)	7(2.9%)		
Farmer	3(1.2%)	4(1.6%)		
Total	23(9.4%)	29(11.8%)		

Note: X^2 = stand for Chi-square

DISCUSSION

In this study, we investigated the prevalence of *candida albicans* in diabetic patients attending the Sokoto Specialist Hospital and the Maryam Abacha Women's and Children's Hospital, Sokoto, Nigeria. It showed a prevalence of 9.4%, which contradicted the findings of Shinkafi *et al.* (2021), who recorded a 21.7% prevalence in Zamfara, Nigeria. However, the prevalence obtained in this study was similar to the 8% obtained by Bashir *et al.* (2022) and lower than the 45.7% reported by Sule *et al.* (2020), respectively, in Kano, Nigeria. The finding was also lower than the 20% observed by Karwiti *et al.* (2022) and the 37.2% reported by Hussain *et al.* (2022), an Indonesia study conducted in Baba Ramrik Singh Hospital Calcutta. These differences in prevalence may be related to differences in the type of technique used to isolate the pathogen, differences in patient characteristics, and the presence or absence of symptoms in the study population. This finding was similar to a study showing that candiduria is more common in pregnant women (Tsaku *et al.*, 2019). This study can be compared to that of Sobel (1997), who found several risk factors, including urinary device use, diabetes, antibiotic use, immunosuppressive treatment, age, and gender, are associated with the development of Candida in urine.

In another study by Rodrigues *et al.* (2019), they isolated *Candida albicans* with the highest isolation frequency (20 isolates) but 5 isolates for non-albicans species. On the other hand, Abdullahi and Danyaya (2017) only isolated 3 *Candida* species: *Candida dubliniensis*, *Candida troppicalis,* and *Candida albicans,* which had the largest isolation rate 29 (80%).

The gender distribution of the isolates showed that females were at higher risk than males, with an isolation rate of 14 (5.7%) and 9 (3.7%), respectively. This was inconsistent with the study conducted by Falahati *et al.* (2016) from 76 urine samples from diabetic women, who reported abundant spores/pseudohyfa in 16 samples (21.05%). The results of this study were comparable to the work performed by Sule *et al.* (2020), who reported an 82.1% higher prevalence in women at Aminu Kano Teaching Hospital, Kano, Nigeria. Therefore, women with diabetes are at risk of *C. albicans* infection.

This study was consistent with the view of Sopian *et al.* (2016), who suggested that because the female urethra is shorter than the male urethra, women are more likely to develop Candidiasis than men. The urethra used to carry urine in women is very short, only 4 cm, while in men it is longer, 20 cm, which is the length of the penis. Women are more likely to develop Candidiasis than men due to anatomical variations and the high frequency of diabetes in women.

This study showed that patients over 55 had a higher rate of *C. albicans* infection than other age groups, with a prevalence rate of 7.3%. This contradicted the findings of Sule *et al.* (2020) and Adebiyi *et al.* (2015), where the prevalence of 37.5% and 25.4% in diabetic patients in Kano and Ibadan, Nigeria, were recorded. However, this does not correspond to the results recorded by Akbar *et al.* 2022 at Bhayangkara Hospital in Palembang, where 3 out of 26 samples collected from patients over 60 years old were positive for *C. albicans* infection. The body's physiology can become less functional as we age, and many diseases, including diabetes, do not become apparent until later in life. Due to a weakened immune system, elderly people are at risk of acquiring Candida infection.

The findings show that the Hausa ethnic group was the most infected in this study area, with a rate of 7.8%. This might be because the Hausa people have the highest population in the study area. This result disagreed with the results of Tang *et al.* (2015), who recorded a prevalence of 20.6% but agreed with the 9.2% prevalence reported by Rodrigues *et al.* (2019).

It was observed that housewives had the highest prevalence of 3.7% compared to other occupation-related factors. This may be related to their exposure to various infections during pregnancy when their normal vaginal microbiota is suppressed. This contradicted the work of Couhan *et al.* (2019), who reported a prevalence of 16.7%, but closely consistent with the findings of Rodrigues *et al.* (2019), who recorded a prevalence of 7.9%.

CONCLUSION

This research shows a prevalence of 9.4% in diabetic patients. The results show that women are the most vulnerable compared to men, with rates of 5.7% and 3.7% respectively. The incidence rate was higher in the age group ≥ 55 years (7.3%), while in the age group 35 – 49 years, it was 0.4%. The findings also showed that Hausa and Fulani had 7.8% and 1.6% infection rates, respectively. This could be attributed to differences in their population and foods consumed by each ethnic group. It is necessary to continue to evaluate the rate of *Candida albicans* infection in diabetic patients in hospitals and the community.

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