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ORGAINAL RESEARCH ARTICLE

PREVALENCE OF ORAL CANDIDIASIS AMONG DIABETICS - NON DIABETICS PATIENTS AND EVALUATETHE CONTRIBUTION OF RISKFACTORS IN IBB CITY.

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Abstract

Studies of oral fungal have indicated that prevalence of Candida was significantly higher in diabetics both in healthy controls and complete denture wearers compared to non -diabetics. The present investigation was carried out in the department of medical microbiology and clinical samples were collected through regular visits to three main hospitals Al-Noor, Al-Amean, Al-Thawra and diagnosis was in the ALFA medical laboratory in Ibb City during period from January into November 2016 .In the current study 70 oral swabs samples were collected from surfaces of the upper of oral cavity and denture of all patients and then placed into a test tube containing 5ml subouraud's dextrose broth. The positive sample was processed for Gram's staining, Germ tube test, Chlamydospores test, Carbohydrate utilization medium examination and Chromagar test was made for differentiation between *Candida* a species .The results in this study showed that prevalence of *Candida* oral infection in Ibb City was 36(52%) positive with oral candidiasis out of 70 samples studied while, 34(48%) of patients studied were negative with oral candidiasis. The results also showed the distribution of oral *Candida* infection among diabetes and non-diabetes patients was 29(41%) and 7(11%) respectively, this results concordant with many previous studies. *Candida albicans* was the most prevalent types of *Candida* a species in this study. The results in current investigation showed a significantly relationship between crews users, diabetes patients, age and oral candidiasis. Through, our study we recommending that many future studies with advanced diagnostics methods have to do for conformed our results.

Introduction:-

Oral Candidiasis (also known as oral thrush) is a common opportunistic mycosis (yeast infection) of *Candida* species on the mucous membranes of the mouth (Alves, *et al*., 2007). *Candida albicans* is the most common species of yeast isolated from patients with oral Candidiasis (Peters, *et al*., 1966 ;Aly, *et al*., 1995). Oral Candidiasis is an opportunistic infection of the oral cavity it affects various sector of the world population irrespective of age or health

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statusabout 90% of AIDS patients suffer from oral cavity or esophageal Candidiasis at some stage of the disease (Bergendal, *et al.,* 1979).The incidence of *Candida albicans* isolated from the oral cavity has been reported to be 50 to 65% of people who wear removable dentures and around 90 to 95% of patients with acute leukemia undergoing chemotherapy, with HIV patients and patients receiving radiation therapy for head and neck cancer (Akpan and Morgan, 2002).

Oral candidiasis is caused by an overgrowth by a yeast-like fungus, *Candida* the commonest ones are *C.albicans* **,** *C.tropicalis*, *C.glabrata*, *C.pseudotropicalis*, *C.guillierimondii*, *C.krusei*, *C.lusitaniae*, *C.parapsilosi s*, and *C. stellatoidea*. Many previous studiesreported that *C. albicans*, *C. glabrata*, and *C. tropicalis* represent more than 80% of isolates from clinical infection(Bai, *et al*., 1995). It is well established that diabetes mellitus is a predisposing factor to fungal infections **e**especially those caused by *Candida* species (Darwazeh, *et al*., 1991). Several studies have reported that the prevalence of yeast carriage among patients with diabetes could reach up to 54% and that *C.albicans* could account for 25-69% of the isolates (Epstein, *et al*., 1980). Oral colonization with *Candida* species occurs more frequently in diabetic patients compared with non-diabetic individuals (Bergendal, *et al*., 1979). In some studies the oral carriage rate of *Candida* has been estimated at around 80 % with diabetic individual (Loiselle, *et al.,* 1964). Studies of oral fungal have indicated that prevalence of *Candida* was significantly higher in diabetics both in healthy controls and complete denture wearers compared to non-diabetics (Abu-Elteen and Abu-Alteen, 1998;Mousavi, *et al*., 2012). Oral thrush (oral Candidiasis) symptoms include: A nasty or bitter taste redness or bleeding inside the mouth, creamy white colored patches (lesions) in the mouth (cheeks, lips, tongue or the back of the mouth and angular cheilitis) (Nanetti, *et al.,* 1993). Local risk factors have been associated with an increased oral Candidiasis prevalence and carriage includes sex ,age , xerostomia, tobacco smoking, denture wearing and poor oral hygiene maintenance (Bastiaan and Reade, 1982 ;Peleg, *et al.,* 2007). Systemic diseases such as poorly-controlled diabetes mellitus, acquired immune deficiency syndrome and renal disorders have also been associated with an increased oral *Candida* carriage, which make immunosuppressed patients more susceptible to develop oral Candidiasis as compared with their systemically healthy counterparts (Tapper-Jones, *et al.,* 1981). The Aims of Study was to determine the prevalence oral Candidiasis among both diabetics and non–diabetics patients in Ibb City. Evaluatethe contribution of riskfactorswiththe prevalence and distribution of *Candida* species with oral Candidiasis.

Materials and methods:-

The present investigation was carried out in the department of medical microbiology whiletheclinical samples were collected through regular visits to the combination of the three main hospitals Al-Noor, Al-Amean and Al-Thawrahospital.Diagnosis of sampeswas in ALFAlaboratories in Ibb City during period from January into November 2016. In the present investigation 70 of oral swabs samples were collected from oral cavity and the tissue surface of the upper denture of all patients each swab was placed into a test tube containing 5 ml of Subouraud's Dextrose broth then covered the tube with cotton and then placed it in the incubator for 48-74 hours at a temperature of 37Ċ.

The samples were brought to the Department of Microbiology Laboratoryin Ibb University & diagnosed them in ALFALab and culture media was processed for prepared Subouraud's Dextrose Agar (SDA) and Yeast Extract Peptone Dextrose Agar (YEPDA).Culture media was sterilizedin autoclave at 121Ċ for 20 minutes and the atmospheric pressure 1ap. The composition was as following: Yeast extract10 g, Peptone 20 g ,dextrose 20 g, agar 15 g, distilled and water 1000 ml . Petri dishes was streaked and incubated within 48 h at 37 Ċ. (Cartwright, 1976; Fenn, *et al*., 1999; Freydiere, *et al.,*2002 ; Roberts, *et al.,* 1978). The positive clinical samples were stained by Gram's staining and then examined by the germ tube test. Specialized chlamydospores formation test on rice meal agar and Carbohydrate utilization medium was also made for differentiation of yeast (Powell, *et al.,*1998 ;Willinger, *et al*., 1999). In the present study, a survey was conducted by taking a brief history of the patients attending in Ibb hospitals, according to the questionnaire which includes socio-economic conditions and demographic history such as name ,address was optionally for the patient to reveal, age ,number of people in the family, educational level illiterate or of literacy, occupation and economic status. Patients history of disease was taken with diabetes mellitus ,gingivitis ,denture wearing and other diseases. Demographic characteristics of the patients and the risk factors was statistically analyzed to ensure homogeneity between the groups by analysis of several proportions used to compare the percentage of different species of *Candida* among experimental groups. The percentage between diabetics and non-diabetics and relation with the denture **w**ear also taken.

Results & Discussion:-

Diabetes is rapidly becoming a major public health problem worldwide. The prevalence of oral *Candida* infections in the current study among patients with diabetes mellitus and non-diabetes in Ibb City is concordance with numerous previous studies, which have shown that diabetes mellitus is a major predisposing factor to oral candidiasis and which have all also indicated that diabetes mellitus enhances *Candida* colonization and proliferation in oral cavity. The results in our study showed the prevalence of oral *Candida* infection in Ibb City as explained in the (Figure 1) 36 (52%) out of 70 from the patients studied was positive with oral candidiasis and we confirmed that by streaked the isolates on the YEPDA media and gram positive yeast cells as showed in the (Figure 2 , Figure 3). while, 34(48%) from patients was negative. The percentage of prevalence oral *Candida* infections among patients in the current study was concordance with numerous previous studies which was very close with the 53.2% reported from Iran, 51.25% India but lower than 61.8% reports from Ethiopia, 58.3%,Mexico and Sao Paulo, in Brazil (66.4%) (Katiraee, *et al.,* 2010). Because the diabetes and high sugar levels in blood lead to better conditions for the yeast to grow & poor hygieneof mouth remaining risk factors in case of oral candidiasis infection (Agwu, *et al.,* 2011). Therefore, it is reasonable to suppose that prevalence of patients of oral candidiasis in our study was more susceptible because changes in the oral environment that can predispose or precipitate oral candidiasis. In additionto wearing dentures &health conditions, such as antibiotics, corticosteroids, dry mouth (xerostomia), nutritional deficiencies, and immune suppressive diseases and therapy which lead to immune system weakened may plied important role in prevalence of oral candidiasis. A higher prevalence percentage in women 20 with (29%) were observed in the present investigation (Table 1) while, 16 with (23%) was in men and which concordance with other ptriviosstudies results. It has been found that elderly women presented more oral lesions than men may lead to the hormonal factor and the great incidence of iron deficiency in women could be responsible for that disparity. In addition, this difference can be explained by the fact that women seek dental treatment at a higher rate than men (Mousavi, *et al*., 2012).

In the (Table 2) results showed the percentage of distribution of oral candidiasis with diabetes and non-diabetes patients whereas, was 29 (41%) and 7 (10%) respectively. The risk of acquiring oral candidiasis was significantly greater among diabetics than non-diabetes patients in this study. The a strong relationship between diabetes and oral candidiasis has been extensively studied in the many literatures and which explained that **y**east adhesion to epithelial tissues surfaces are recognized as an essential first step in the process of *Candida* colonization of oral cavity and subsequent infection. Salivary glucose levels in diabetic patients favors yeast growth owing to increased numbers of available receptors for *Candida* (Yarahmadi, *et al.,* 2002). This results in the current investigation are in agreement with numerous previous studies, which have all indicated that diabetes mellitus enhances *Candida* colonization and prevalence (Tapper-Jones, *et al.,* 1981) have shown that 42% of healthy non diabetics harbor *C. albicans* in their mouths compared to 60% of diabetics patients. Other suggested that 16.2% of the controls and 40.2% of the diabetics carry *C. albicans* in the mouth (Katiraee, *et al.,* 2010). Consequently, buccal cells from diabetic patients have shown an increased adherence of *C. albicans* compared with buccal cells from non-diabetics. In addition to, micro vascular degeneration found in histological examination of diabetic patients may also predispose to *Candida* colonization and making them more susceptible to infections. Another host factor that may promote the oral carriage of *Candida* in diabetics is the possible defects in *Candida* activity of neutrophils, particularly in the presence of glucose. Reduced salivary flow, associated with diabetes, may also play a role in *Candida* colonization and consequently in the pathogenesis of oral candidiasis in these patients (Akpan and Morgan, 2002). The distribution of *Candida* species obtained from 70 patients studied in Ibb City we found *Candida albicans* was the most prevalent species 29 out of 36 with (80%) from positive clinical samples as shown in the (Table 3) while, *Candida dubliniensis*found with 6 (17%) and *Candida tropicales* was 1(3%) we conformed this results by used differentiation tests such as germ tube test ,rice meal agar test and carbohydrates fermentation test as showed in the (Figure 4,5 and 6) this results are very similar with other previous studies and this indicate that*Candida albicans* has the ability to adhere to mucosal and denture surfaces, which is considered to be the first step in the pathogenesis (Mousavi, *et al*., 2012). The ability of *Candida albicans* to changes in the host environment may respond to increasing the number of colonies forming units, and invade tissues and causing infections that require care privacy spread because of cooperation with the dental prosthesis**.**(Table 4) The results showed the relationship between prevalence of oral candidiasis and age groups we found older group (65-60) was significantly 13 (19%) because are more susceptible to opportunistic diseases caused by microorganisms, which is due to the decline in the ability of the immune system and systemic diseases**.** In addition to the main reason may be behind this results the fall of the teeth and dry mouth because of inflammatory periodontal tissue support that. Thus, the results in the current investigation showed that adults are more susceptible to oral candidiasis and this concordance with many previous studies. In the current study we also investigated the relationship between prevalence of oral candidiasis

and the wearers of artificial dentures, we found the number of who wear artificial crews was 25 (36%) from positive clinical samples studied (with oral candidiasis) while, those who do not wear artificial dentures was 11(16%) from positive clinical samples (with oral candidiasis) as shown in (Table 5). A significantly higher incidence of oral *Candida* infection were found with diabetic patients and wearing removable dentures because the presence of a removable denture may decrease the salivary pH and saliva flow rate and impede the mechanical cleaning of the soft tissue surfaces by the tongue. In addition, denture induced trauma may reduce tissue resistance against infection because of the increase in permeability of the epithelium to soluble *Candida* antigens and toxins. Moreover, the tissue surface of the acrylic resin denture acts as a reservoir that harbors microorganisms, enhancing their infective potential and aggravating a previously existing condition. In addition to these factors, the observed high prevalence of oral candidiasis in the number of users of denture because dentures can evolve acidic and anaerobic environment in the oral mucosa promotes yeast proliferation, while those who do not wear the crews have the appearance of fungus mouth is remarkably dramatically (Ozturkcans, *et al*.,1993 ; Peleg, *et al*., 2007). The results in this investigation showed there is no clear correlation between prevalence of oral candidiasis infection and presence of gingivitis (Table 6), this may inconsistent with other previous studies. Nevertheless, gingivitis is a chronic inflammatory disease characterized by the formation of a periodontal pocket, loss of connective tissue and alveolar bone resorption, which may sometimes result in tooth loss. The contribution of smoking in the prevalence of oral candidiasis was also evaluated and results in (Table-7)showed there is no significantly correlation in this current investigation. Thus, suppose in comparison with previous studies conducted in Jordan, furthermore, in comparison with studies conducted in Lebanon, United States or the United Kingdom, there are seemingly, difference and inconsistent between results of these studies and our investigation results (Akpan and Morgan, 2002).

Figure1:-Prevalence of oral cavity candidiasis between clinical samples studied in Ibb City.

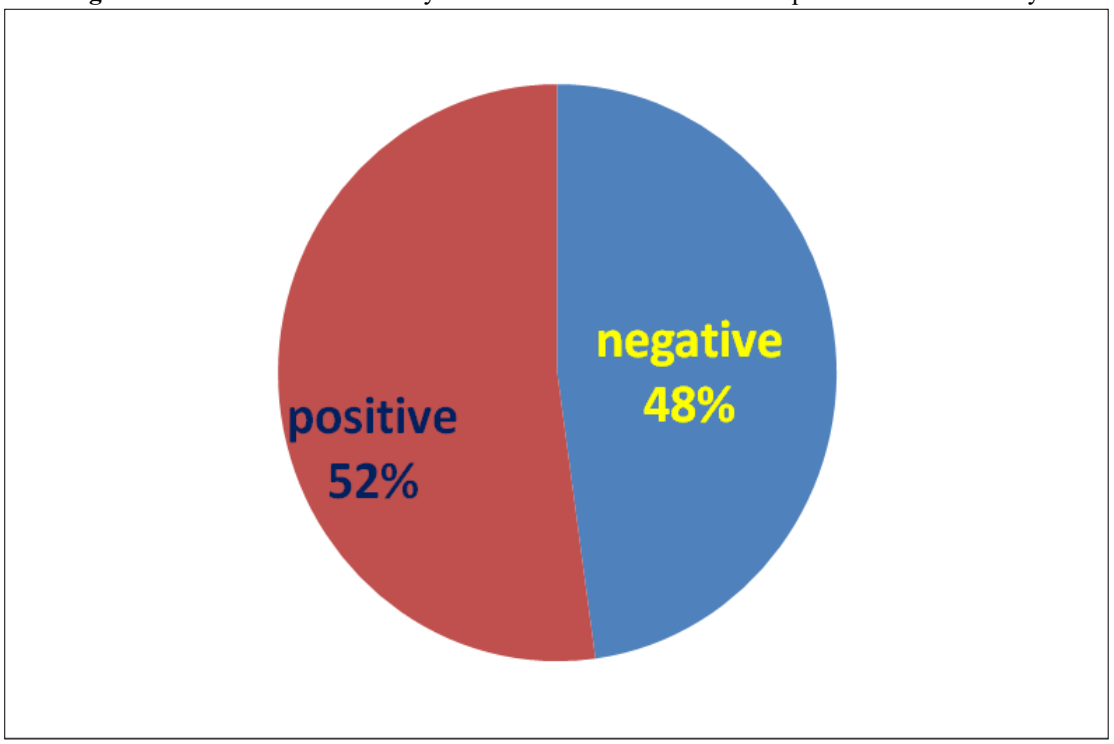

Table 1:-Prevalence of oral cavity candidiasis between clinic samples studied based on sex in Ibb City.

Prevalence of oral Candidiasis						
Sex	Negative patients		Positive patients		Total	
	Number	Percentage	Number	percentage	Number of patients studied	%
Man	15	21%	16	23%	31	44%
Women	19	27%	20	29%	39	56%
Total	34	48%	36	52%	70	100%

Table 2:-Distribution of oral *Candida* carriage with diabetes and non-diabetes patients in Ibb City.

Case type	Positive patients	
	Numbers of cases	%
Oral candidiasis with diabetes mellitus.	29	41%
Oral candidiasis with Non-diabetes mellitus patients.	7	11%
Total of Oral candidiasis	36	52%

Case type	Negative patients	
	Numbers of Cases	%
Oral candidiasis with diabetes mellitus.	13	18%
Oral candidiasis with Non-diabetes mellitus patients.	21	30%
Total of Oral candidiasis	34	48%

Table 3:-Number and prevalence of different *Candida* species isolated and identified from all oral cavity samples studied.

Type of Candida	Number	%
C.albicans	29	80%
C.dublinieosis	6	17%
C.tropicales	1	1 3%
Total	36	100

Table 4:-Prevalence of oral candidiasis in patients based on age Ibb City.

Distribution of all clinical samples studied based on age.						
Age group (in years)	% age of cases positive for candidiasis in each age group		% age of cases negative for candidiasis in each age group		Total	
	Frequency	%	Frequency	%	Frequency	%
60-65	13	19%	7	10%	20	29%
45-55	7	10%	3	4%	10	14%
35-40	4	6%	6	9%	10	14%
25-30	7	10%	12	17%	19	27%
15-25	5	7%	1	1%	6	9%
15>	0	0%	5	7%	5	7%
Total	36	52%	34	48%	70	100%

Table 5:-Prevalence of oral cavity candidiasis and it relationship with the denture wearing.

Artificial crews used.	Oral cavity with candidiasis		Oral cavity without candidiasis		Total	
	Frequency	%	Frequency	%	Frequency	%
presence	25	36%	2	3%	27	39%
Absence	11	16%	32	45%	43	61%
Total	36	52%	34	48%	70	100%

Table 6:-Prevalence of oral cavity candidiasis and it relationship with the gingival diseases.

Gingival diseases (gingivitis)	Oral cavity with candidiasis		Oral cavity without candidiasis		Total	
	Frequency	%	Frequency	%	Frequency	%
Yes	18	26%	20	28%	38	46%
No	18	26%	14	20%	32	54%
Total	36	52%	34	48%	70	100%

Table 7:-Prevalence of oral cavity candidiasis and it relationship with the smoking.

Smoking peoples	Oral cavity with candidiasis		Oral cavity without candidiasis		Total	
	Frequency	%	Frequency	%	Frequency	%
Yes	4	6%	3	4%	7	10%
No	32	46%	31	44%	63	90%
Total	36	52%	34	48%	70	100%

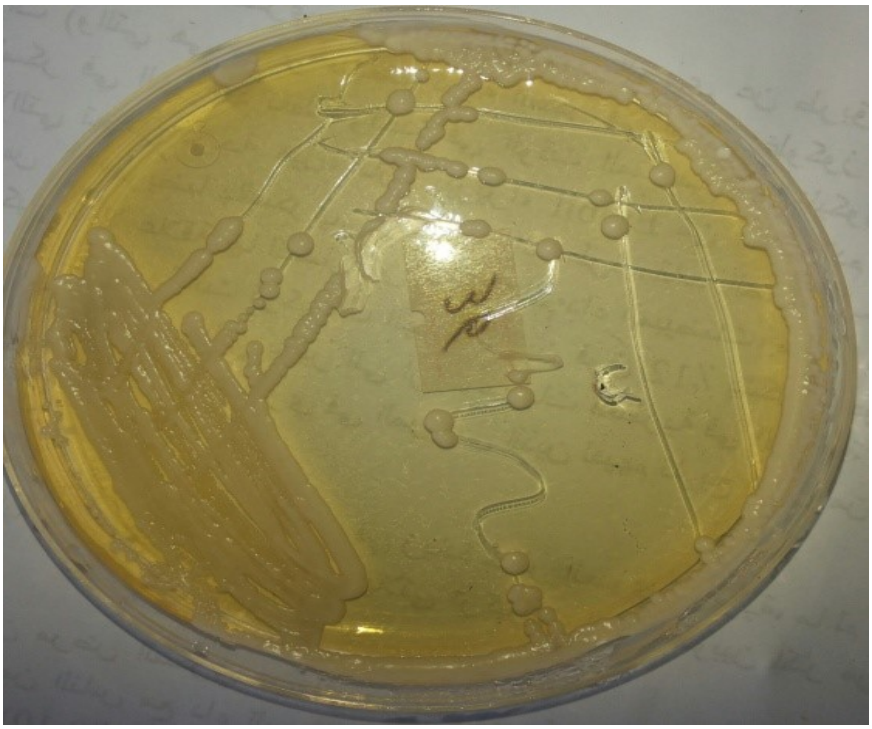

Fig. 2:-48-h-culture growth of *Candida albicans* on SDA plate isolated from oral cavitypositive patient.

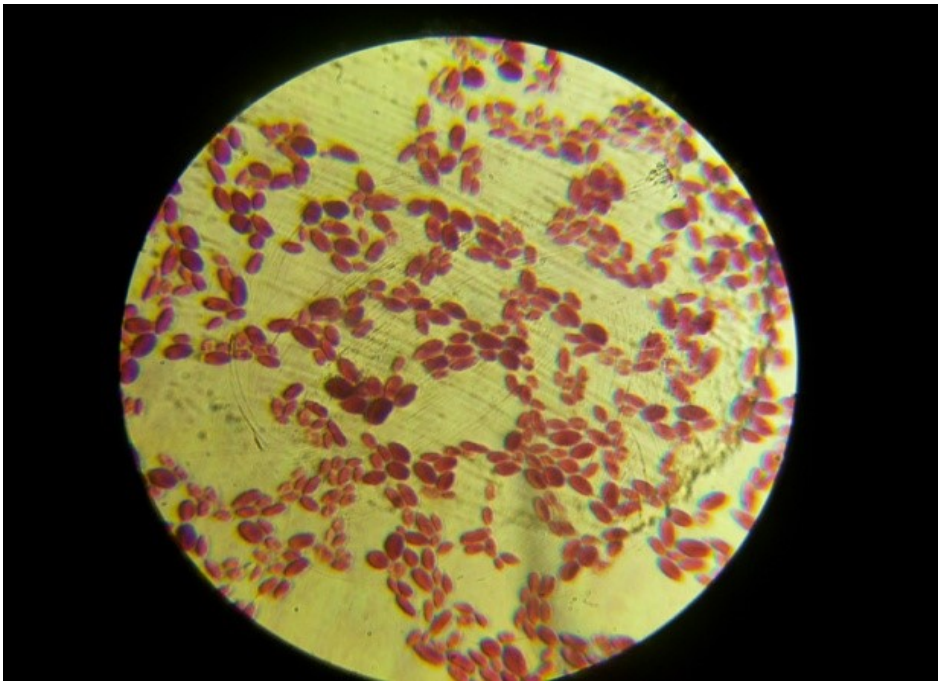

Fig. 3:- Gram positive showed of budding blastoconidiafor*Candida cells.* (10X x 100X).

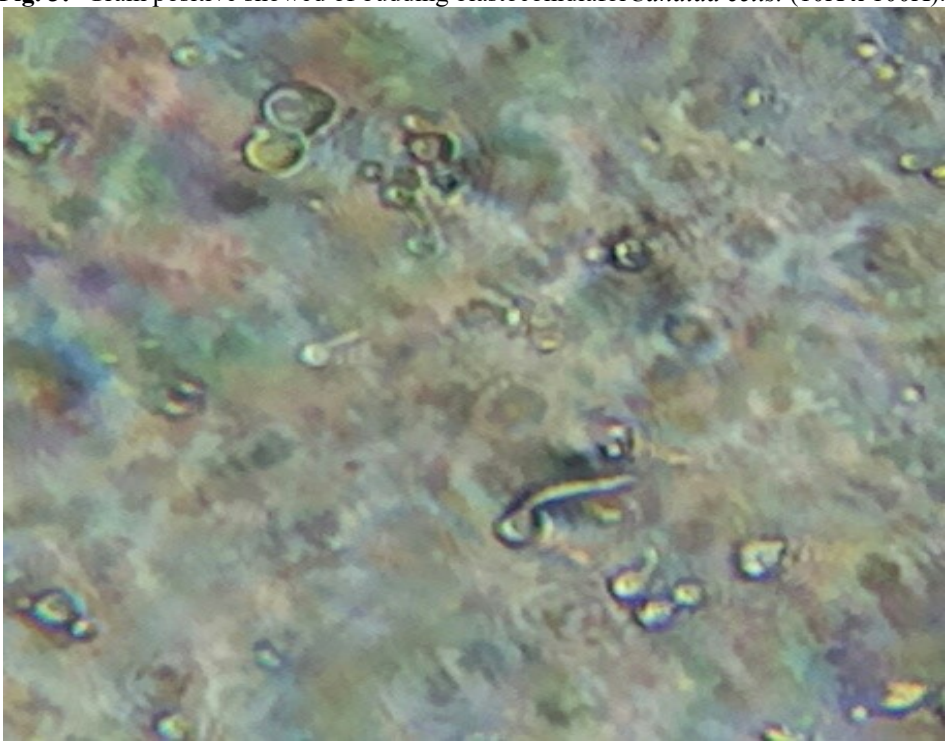

Fig. 4:-Photomicrograph of germ tube test in *Candida albicans*(10X x 100X).

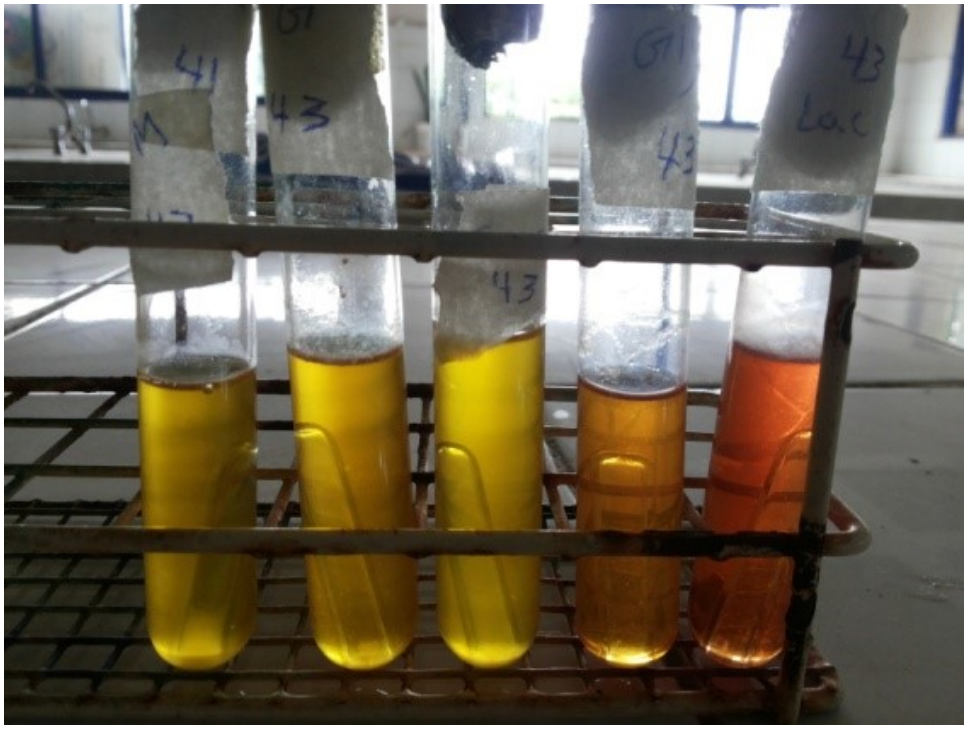

Fig. 5:-Sugar fermentation test for identification of *Candida* species.

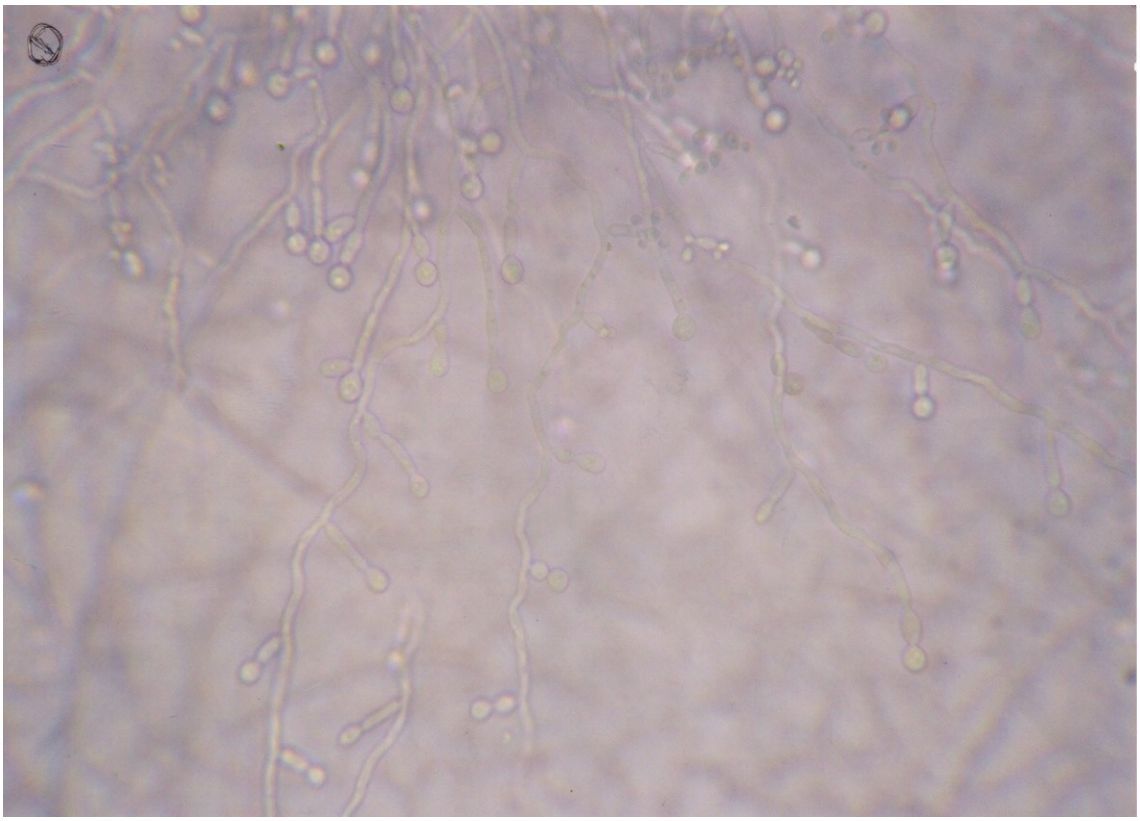

Fig.6:- Growth of *Candida albicans* on rice meal agar showing of Chlamydospores.

Conclusion:-

It is clear that diabetics are more susceptible to oral candidiasis than non-diabetics. Furthermore, diabetic and denture wears are at high risk of being infected. *C. albicans* is the most prevalent among all *Candida* spp. as the cause of oral Candidiasis in Ibb City. The prevalence of oral candidiasis obtained in our sample was (52%), confirming that variability, which could depend on risk factors**.** Excellent oral hygiene, including brush in gland flossing of the teeth twice daily and maintenance of adequate intraoral moisture, is critical in the prevention of candidiasis recurrence in the susceptible patient. In clinical terms, equal attention should be given to both local and systemic predisposing factors to suppress the *Candida* density and hence reduce the risk of oral candidiasis in diabetes mellitus.

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