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Diagnosis of Candidiasis and Hepatitis C virus in Thalasemia Patients

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Abstract--- *This research was performed with 96 male and female patients aged from one to fifty years, divided into 7 β-thalassemia patients with HCV infection and 89 β-thalassemia patients without HCV infection, who visited the Thalassemia Center at Al- Zahra Teaching Hospital in Al–Najaf Iraq for disease management and 90 volunteer stable patients as a control group. Sample collection was conducted between April 2019 and the end of August 2019. This analysis tends to be 48 (48 percent) that offer Candida spp a good outcome. Sabouraud dextrose agar (SDA) was cultivated from all the collected samples. The good finding cultivated on Chromagar that contained most of the isolates was also Candida albicans 43.75%, led by Candida parapsilosis 39.58%, Candida galabrata 10.41%, Candida tropicalis 2.08% and 4.16% of unknow species. Some virulence factors in C.albicans such as: germ tube and the formation of biofilm were established in the present analysis. The findings showed that all C.albicans isolates were able to create germ tube and development of biofilms.* ***Conclusion:*** *There is a association between HCV- thalassemia patients that showed substantial difference at p- 0.05 between Infected and non- classes. Once cultivated on Chrom agar, the largest abundance of C. albicans than other animals.*

Keywords--- *Germ Tube, Biofilm and Chrom Agar.*

I. INTRODUCTION

A group of hemoglobin synthesis genetic disorders characterized by a disruption in the development of the globin chain which causes varying degrees of anemia, ranging from negligible to life-threatening[1].

The word "Thalassemia" applies to a category of hereditary blood disorders defined by decreased production of one of the two forms of polypeptide chains (α or β) containing the typical adult human hemoglobin molecule (HbA, α2 β2) resulting in decreased filling of the red cells with hemoglobin and anaemia[2].

Blood transfusion relates to the continuation of long-life therapeutic approaches in β-thalassemia disease but remains accomplished with numerous infections, most of which are HCV for many health care systems due to continuous migration of people, especially from countries around the Mediterranean Sea, who are endemic to β-thalassemia causing increased incidence[3].

Post-transfusion hepatitis (PTH) is primarily induced by hepatitis C virus and is deemed a significant concern for large patients with β-thalassemia[4]. Until conducting screening procedures in infected blood, 60-80 percent of the main patients with β-thalassemia had HCV infection[5].

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Based on the most new research, while *C. albicans* are the most prevalent species causing invasive candidiasis in adults, several studies have shown that *C. parapsilosis* is even out of *C.albicans* in specific patient classes, particularly in neonates[6]. Candida bacteria are recognized as one of the most important sources of human infection. Candidacies are a natural yeast-like fungal infection[7].

II. MATERIALS AND METHODS

The study was carried out at the Department of Biology Bacteriology and Molecular Labs, Faculty of Science, Kufa University, Iraq. Patients suffering from beta-thalassemia but omitted patients with alpha-thalassemia or other genetic disease were involved.

Patients and Control Group

Beta-thalassemia patients diagnosed with an acquired blood disorder center at Al-Zahra Teaching Hospital in AL- Najaf between April 2019 and the end of August 2019; 96 patients aged between (one to fifty years) years.For each topic, physical and clinical assessments were performed, and the specifics were recorded in a data sheet. This research followed the values of the Ministry of Health and Al-Zahra Teaching Hospital.

Collection of Samples

For both subjects, three milliliters for venous blood sample were obtained. A tourniquet was applied directly to the skin around the wrist, the skin over the vein was sterilized with 70 percent of patients 'ethyl alcohol and control group prior to collecting of blood, The blood samples were then moved to the Gel tube for serum isolation, the blood was held at room temperature for coagulation for about 30 minutes and then centrifuged at 3000 g for 2 minutes, then the serum was extracted in three repeaters in a sterile appendrophic tube and kept frozen at -20 C for ELISA (IgG anti-HCV) processing.

Oral Cavity Samples

The samples were taken in Beta-thalassemia patients by sterilized cotton swabs from the oral cavity and delivered to the SDA laboratory and culture as soon as possible, then incubated at 37C ° for 24-48 hr. for clear development of *Candida spp*. Settlements [28].

HCV Antibody ELISA Test kit: by automated ELISA / Japan

Statistical Analysis

Statistical analysis has been carried out using a system / version 17 of the Statistical Software for Social Science (SPSS) and Microsoft Office Excel 2007. Statistics have been measured as mean ± S.D. P-value was found important if it is below 0.05. Variance Analysis (ANOVA) was also performed.

III. RESULTS AND DISCUSSION

Demographical Distribution of Thalassemia Patients

The following findings were reported for ninty six patients visiting the inherited blood disorder center in the province of AL-Najaf and ninty patients as a control study:

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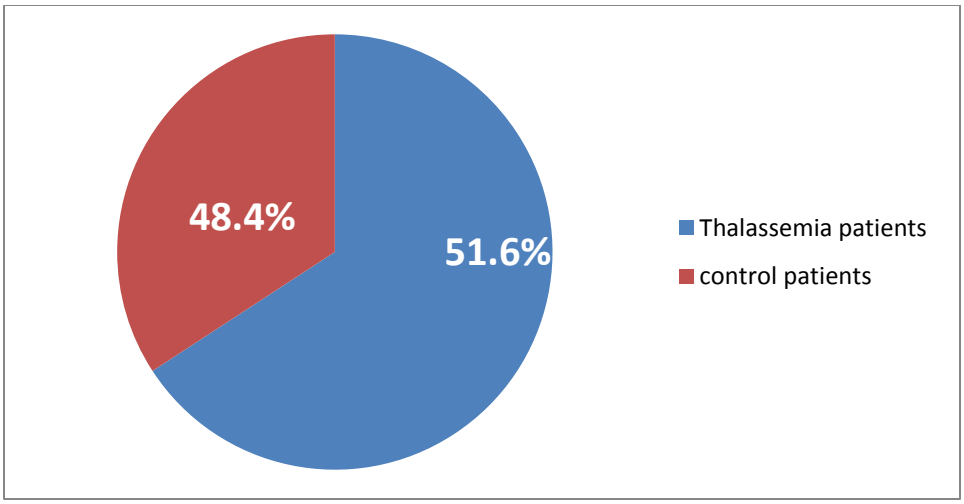

Figure 1: Distribution of the Thalassemia Patients with Control

HCV Infection among Thalassemia

Of 96 patients with thalassemia, 7 (7.3%) were reported positive for antibodies to HCV. The remainder is seronegative 89 (92.7 %).

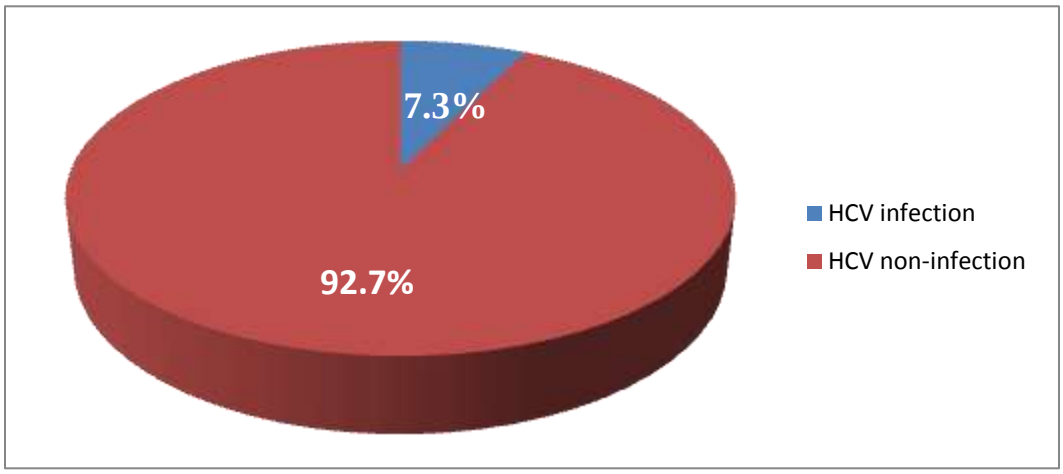

Figure 2: HCV Sero-positivity Results in Thalassemic Patients

In this analysis, Figure (2) indicates that the number of positive anti-HCV antibody cases was 7.3% small. It is a comparatively small level, bearing in mind that all blood collected is tested daily for HCV at all thalassemic centres, and the main reason for this broad variety of HCV infection levels could be the form of testing methods used, Check exposure or the development of laboratory techniques used to diagnose HCV infection. Another reason for the wide spectrum of HCV prevalence in thalassemia patients could be the elevated prevalence of HCV in the patient community as a whole, comparable with [9 & 27].

Hepatitis C Virus Distribution According to Age and Gender

Table (1) in this study tends to be the relationship between gender and age Cross tabulation according to HCV infected or non-infected that finds female infected with HCV to be more frequent than male infected with HCV and

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spread across the following age scale, 21-30 and 1-10, although 11-20 revealed that male more than female is 100% female by sex and age. This finding is P-value discrepancy of magnitude 0.05that show in table(5).

Table 1: Distribution of HCV Infection among Thalassemia Patients According to Age and Gender

Gender * age Crosstabulation							
Age groups		Infected			Non-infected		
		Male	female	Total	male	female	total
1-10	Count	0	1	1	17	21	38
	% within Gender	0.0%	20.0%	14.3%	44.7%	41.2%	42.7%
	% within age	0.0%	100.0%	100.0%	44.7%	55.3%	100.0%
11-20	Count	2	0	2	18	19	37
	% within Gender	100.0%	0.0%	28.6%	47.4%	37.3%	41.6%
	% within age	100.0%	0.0%	100.0%	48.6%	51.4%	100.0%
21-30	Count	0	4	4	1	10	11
	% within Gender	0.0%	80.0%	57.1%	2.6%	19.6%	12.4%
	% within age	0.0%	100.0%	100.0%	9.1%	90.9%	100.0%
41-50	Count	0	0	0	2	1	3
	% within Gender	0	0	0	5.3%	2.0%	3.4%
	% within age	0	0	0	66.7%	33.3%	100.0%
Total	Count	2	5	7	38	51	89
	% within Gender	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% within age	28.6%	71.4%	100.0%	42.7%	57.3%	100.0%
Chi-Square Sig.		7.000[a] 0.03			6.382 0.094		

A significant difference at p-value 0.05

Thalassemia patients infected with HCV may be attributed to the number of blood units transfused as patients grow older due to growth, the development of antibodies to red blood cells and the possibility of developing hypersplenism, or the possibility of exposure to infected blood or increased hospital admission with increased potential for exposure to infected devices or material, This may be explained by growing the chance of exposure to bloodborn viruses. Or by increasing patient admission frequency with enhanced probability of access to contaminated system or substance, as HCV infection is predominantly nosocomial[10;11].

This finding indicates dissimilar in table (1) with [12] who find 4 males and 2 females with beta-thalassemia contaminated with HCV. The incidence of anti-HCV antibodies in people requiring blood transfusions has reduced significantly after screening of blood donors. Patients younger than 10 years were not HCV-positive, according to an earlier report [13 & 27].

Morphological Identification

Identification Candida on Sabouraud Dextrose Agar Medium

All the samples collected were grown on Sabouraud dextrose agar (SDA), which found 48(48 percent) to give Candida spp a good test. Candida's colonies spp. Colored to yellowish white, rising rapidly in 24-48 hours, colony texture shiny, glittering or dry depending on the organisms. Those findings have been confirmed with [14] as in figure (3).

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Figure 3: Showing Growth of Candida spp. on SDA at 37°C for 48 Hours

Microscopic Identification by Gram Stain of Candida Species

Microscopic analysis is a basic screening procedure for the candida ssp. At the laboratory of advanced mycology / Faculty of Science / University of Kufa microscopic analysis was investigated for diagnosis and research. Each sample was stained with blue stain of lacto- cotton, and microscopically examined.

Once prepared with gram stain, all diagnostic yeast species show positive outcomes, these findings were compatible with [15; 16], Figure (4).

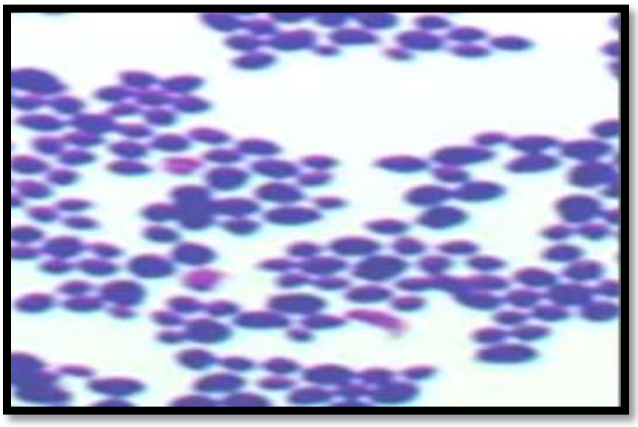

Figure 4: Gram stain of Candida spp. (100X)

Identification of Candida spp. on chrom Agar Medium

Chrom agar is a selective medium for yeast isolation and provides a clear distinction and identification of several Candida spp at the same time [18].

This research has shown that the colonies look C.glabrata dark purple, C.krusei purple with white peripheral (fuzzy) and C.parapsilosis white light pink[18] using chromium agar Candida which is called a differential agar. And C.albicans distinguished by light green color smooth colonies and C.tropicalis dark greenish brown, Figure(5) and Table(2).

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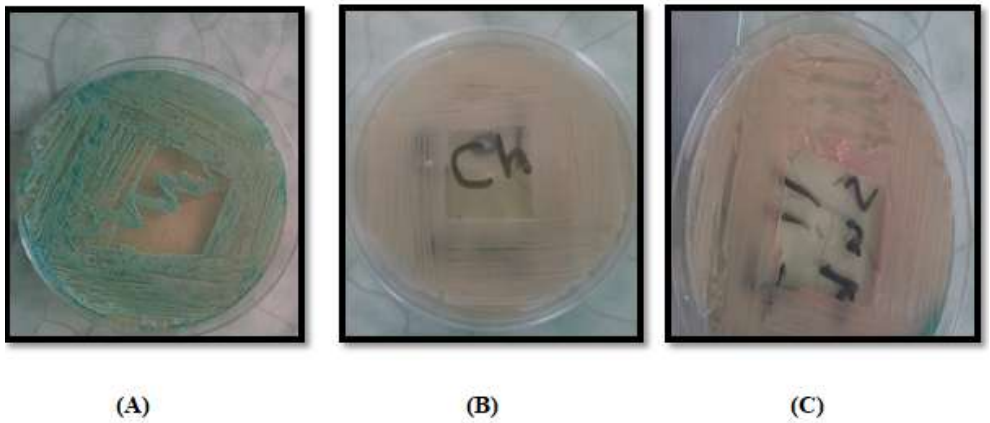

Figure 5: The Colony of A- Candida Parapsilosis, B-Candida Albicans and C- Candida Glaprata on Chrom Agar at 37C for 48 Hours

Frequency of Candida Species

The predominance of *C. albicans* relative to other *Candida ssp* was significant as seen in Table (2) which shows that the plurality of isolates were *C. albicans* 43.75%, accompanied by *C. parapsilosis* 39.58%, *C. galabrata* 10.41%, then *C. tropicalis* 2.08% and 4.16% unknown.

Candida spp, according to various enquiries. In particular, the C.albicans are the most remarkable pathogenic opportunistic fungi that cause nosocomial UTIs[19].

Table 2: Showing Percentages of Candida spp from Isolated Samples According to Conventional Methods

Yeasts spp.	NO. (%)
C.albicans	43.75%
C. parapsilosis	39.58%
C. glabrata	10.41%
C. tropicalis	2.08%
Unknow	4.16%
Total	100%

Virulence factors for Candida Albicans

Test of Germ Tube

This test was a simple method to classify certain Candida albicans by their ability to develop thin, small, tube-like structures called germ tubes when incubated in serum at 37C0 for 2 to 4 hours. Figure (6) demonstrates the effects of this study of the Candida spp strains for germ tube development and anti germ development.In this examination the researcher will be able to differentiate between the pseudohyphae and the germ drain. It referred to daughter cells that elongated to such germ tubes from the mother cell without constriction of origin Figure (6), while constriction of the origin of the mother cells was called pseudohyphae[20]. *C.albicans* have a better capacity to shape a funnel of germs[21].It is stated that the morphological characteristics of *C. tropicalis*-formed germ-tubes vary from those of *C. albicans* in terms of the hyphal width[22].

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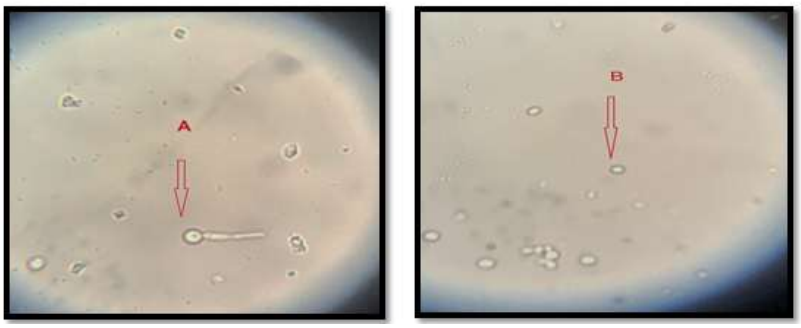

Figure 6: A- Germ Tube Formation of some of C.albicans.B- Non Germ Tube formation of other Candida

Biofilm formation

The findings of this analysis demonstrating the capacity of all isolates of *C. albicans* is positive for Biofilm development. This finding was consistent with [23]. Biofilms have been found an significant virulence factor in infection pathogenesis, as biofilm- microorganisms display an inherent tolerance to antibiotics, disinfectants and clearance through host protection mechanisms[24].

An important factor for virulence is the ability of *C. albicans* to form biofilms on abiotic or biotic surfaces[25]. Recent studies have confirmed the growth of the biofilm in most diseases caused by *Candida spp* [26] As seen in figure (7).

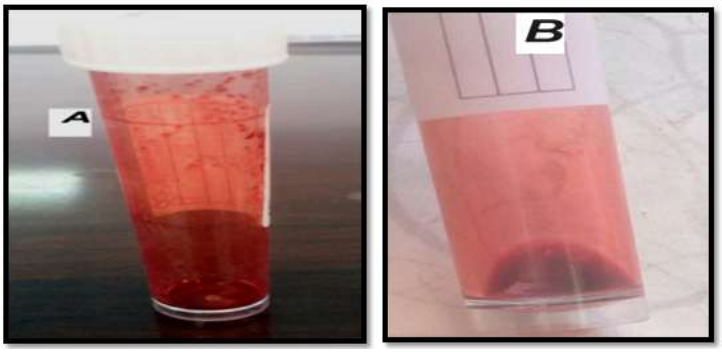

Figure 7: A- Biofilm Formation of C. albicans (Positive); B- Inhibition of Biofilm Formation (Negative)

REFERENCES

[1] AL-Mosawy, w .F.(2017): The-Beta Thalassemia. *Scientific Journal of Medical Research*. 1(1):24-30.
[2] Lichtman, M.A.; Beutler, E. and Seligsohn, U. (2007). Williams hematology, *7th ed, Mc- Grew Hill*, (46): 548.
[3] Javadzadeh SH, Attar M, Yavari M, Savabieh S. (2006). Study of the prevalence of Hepatitis B, C and HIV infection in Hemophilia and Thalassemia population of Yazd. *Sci J Iran Blood Transfus Org;* 2(7): 315-322.
[4] Ataei B, Hashemipour M, Kassaian N, Hassannejad R, Nokhodian Z, Adibi P.(2012). Prevalence of anti HCV infection in patients with Beta-thalassemia in isfahaniran. *Int J Prev Med.,* 3(1):S118-S119.
[5] Vasmehjani A. A.; Yaghoubi S.; Hashemi S. M. A.; Farahmand M.; Adeli O. A.; Taravand A. H. & Beiranvand M.(2018).The Prevalence of Hepatitis B, Hepatitis C, and Human Immunodeficiency Virus Infections among β-thalassemia Major: A Multicenter Survey in Lorestan, West of Iran. *Iran J Ped Hematol Oncol.* Vol 8.No 2, 111-117.

DOI: 10.37200/IJPR/V24I5/PR2020105
Received: 05 Mar 2020 | Revised: 26 Mar 2020 | Accepted: 04 Apr 2020

[6] Lohse, M. B., Gulati, M., Johnson, A. D., & Nobile, C. J. (2018). Development and regulation of single- and multi-species Candida albicans biofilms. *Nature Reviews Microbiology, 16*(1), 19.

[7] Sayyada,G. N.; Shaziia, T.H. and Hahana, U. K.(2010). Use of CHROM agar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource limited setting. *Libyan J Med.* 5: 2144 – 2154.

[8] Ruhnke, M. (2006). Epidemiology of *Candida albicans* infections and role of non-*Candida-albicans* yeasts. *Curr Drug Targets.* 7: 495–504.

[9] Hassanshahi G, Arababadi MK, Assar S, Hakimi H, Karimabad MN, Abedinzadeh M, *et al*.,(2011). Post-transfusion-transmitted hepatitis C virus infection: a study on thalassemia and hemodialysis patients in southeastern Iran. *Arch Virol.* 156(7): 1111 -5.

[10] Majeed, M.N. (2002). Prevalence of hepatitis B and hepatitis C infections among thalassemic children in Najaf city. *Kufa. Med.* 5(1): 192.

[11] Abed, B. A. (2010). Prevalence of hepatitis c virus (HCV) among thalassemia patients in ibn-albalady hospital. *Journal of Al-Nahrain University*, 13 (1): 121-126.

[12] Mohammadi S. and Khodabandehloo M. (2017). Prevalence of Hepatitis C Virus Antibodies among Beta-Thalassemia Major Patients in Kurdistan Province, Iran, *Arch Clin Infect.* 12(3):e62419.

[13] Jafroodi M, Davoudi-Kiakalayeh A, Mohtasham-Amiri Z, Pourfathollah AA, Haghbin A. (2015). Trend in Prevalence of Hepatitis C Virus Infection among beta-thalassemia Major Patients: 10 Years of Experience in Iran. *Int J Prev Med.* 6:89.

[14] Bhavan, P.S.; Rajkumar, R.; Radhakrishnan, S.; Seenivasan, C. and Kannan, S. (2010). Culture and identification of *Candida albicans* from vaginal ulcer and separation of enolase on SDS-PAGE. *Inter. J. Bio.* 2; (1): 84-93.

[15] Iehab, Y. J. (2014). Isolation and Identification of Candida spp.from patients with Oral thrush in AL- Najaf Province and molecular study of some virulence factors. *MSc, Faculty of Science/ University of Kufa.*

[16] Zahraa, M.W.(2016). Phenotypic and molecular characterization of Candida species isolated from hospital acquired infections in Hilla city. *MSc. College of Science for Women/ University of Babylon.*

[17] Sayyada,G. N.; Shaziia, T .H.andHahana, U. K.(2010). Use of CHROM agar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource limited setting. *Libyan J Med.* 5: 2144 – 2154.

[18] Hospenthal, D. R.; Beckius, M.L.; Floyd,K.L.; Horvath, L.L. and Murray, C.K . (2002).Presumptive identification of *Candida* species other than *C.albicans*, *C.krusei* and *C.tropicals* with the Chromogenic medium Chromagar *Candida*. *Annclin microbial Antimicrob.*5:1-5.

[19] Helbig S., Achkar J.M., Jain N., Wang X., Gialanella P., Levi M. and Fries B.C. (2013). Diagnosis and inflammatory response of patients with candiduria. *Mycoses.* 2013; 56: 61–69.

[20] Kim, D.;Shin , W.; Lee , K.; Kim , K. and Park , J. (2002) . Rapid differentiation of *Candida albicans* from other *Candida* species using it unique germ tube formation at 39 C^0. *Yeast.* 19 : 957 – 962 .

[21] Kumar, A.; Sharma, P.C.; Kumar, A. and Negi, V. (2014). A study on phenotypic traits of candida species isolated from blood stream infection and thrir in vitro susceptibility to fluconazole. *Al Ameen J. MED. Sci.* 7(1):83-91.

[22] Criseo, G., Scordino, F., & Romeo, O. (2015). Current methods for identifying clinically important cryptic Candida species. *Journal of Microbiological Methods*, 111, 50-56.

[23] Emily, P.F.; Jeniel, E.N.; Trevor, R.S.; Quinn, M.M.; Aaron, D.H.; Brian, B.T.; David, R.A. and Alexander, D.J. (2011). A Recently Evolved Transcriptional Network Controls Biofilm Development in *Candida albicans.*

[24] Donlan, R.M .(2002). Biofilms: microbial life on surfaces. *Emerg Infect Dis.*8:1–19.

[25] Achkar, M. J., & Fries, B. C. (2010). Candida infections of the genitourinary tract. *Clinical Microbiology Reviews*, 23(2), 253-273.

[26] Chandra, J.; Patel, J.D.; Li, J.; Zhou, G.; Mukherjee, P.K.and McCormick, T.S.(2005). Modification of surface properties of biomaterials influences the ability of *Candida albicans* to form biofilms. *Appl Environ Microbiol.*, 71:8795–8801.

[27] Alsadawi, A. A. & Alnaji, H. (2019). Hepatitis C and IL-6 with 174G/C Gene Polymorphism in β-Thalassemia. *International Journal of Drug Delivery Technology*, 9(4), 617-622.

[28] Loveday, H.P. ;Wilson, J.A.; Pratt, R.J.; Golsorkhi, M.; Tingle, A.; Bak, A.; Browne, J.; Prieto, J. and Wilcox, M. (2014).National evidence based guideline for preventing health care associated infections in NHS hospitals in England. *Journal of hospital infection* 86S1 S1-S70.

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