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Detection of the fungi *Aspergillus* and *Candida* sp. that cause pneumonia and determining the IL 10 response against them

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Abstract :

155 clinical samples (sputum, blood) were collected from patients suffering from various respiratory diseases (asthma, tuberculosis, chest allergies, and chest infections) from the consultation clinic for chest and respiratory diseases in Tikrit. The total number of infected samples was 70, and the number of uninfected samples was 85. The age groups comprised males and females aged between 10 and 65 years. The presence of fungal species belonging to the genus *Aspergillus* was found in 48 samples (68.57%), including *A. niger*, *A. fumigatus*, *A. hiratsukae*, and *A. flavus*. The presence of yeasts belonging to the genus *Candida* was also detected in 22 samples, representing 63.64%, and including *C. albicans*, *C. dublinensis,* and *C. parapsilosis*. Immunological tests were performed on the blood serum of the same people infected with the pathogenic fungi. The immune response was diagnosed with antibodies (IL 10) and a significant increase was observed at the probability level (0.05, 0.01, and 0.001) when comparing the serum of healthy people (20 samples of male and female). We noticed that it was high in the serum of people infected with the fungus, with the exception of the elderly, those with chronic diseases, and those with cancer, as its percentage in their serum was low.

Keywords: *Aspergillus, Candida*, IL 10.

الكشف عن الفطريات *Aspergillus* sp و *Candida* sp المسببة للالتهاب الرئوي وتحديد استجابة IL 10 ضدها

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مستخلص

تم جمع 155 عينة سريرية (بلغم ، دم) من المرضى الذين يعانون من أمراض الجهاز التنفسي المختلفة (الربو، السل، حساسية الصدر، الالتهابات الصدرية) من العيادة الاستشارية للأمراض الصدرية والتنفسية في تكريت. حيث بلغ عدد العينات المصابة بالفطريات المرضية (70) عينة والعينات غير المصابة (85) و كانت الفئات العمرية ما بين (65-10) سنة ذكور وإناث. وجدت الأنواع الفطرية التي تنتمي لجنس *Aspergillus* في 48 عينة (68.57%)، وتشمل *A. niger* , *A. fumigatus* , *A. hiratsukae* , *A. flavus*. كما وتم الكشف عن وجود خمائر تنتمي إلى جنس المبيضات في 22 عينة تمثل 63.64%، ومن بينها *C. albicans* ، *C. dublinensis* ، *C. parapsilosis*. تم إجراء الاختبارات المناعية على مصل الدم لنفس الأشخاص المصابين بالفطريات المسببة للأمراض. تم تشخيص الاستجابة المناعية بالأجسام المضادة (IL 10) ولوحظ ارتفاع معنوي عند مستوى الاحتمال (0.05، 0.01، 0.001) عند مقارنة مصل الأشخاص الأصحاء (20 عينة من الذكور والإناث). وقد لاحظنا ارتفاعه في مصل الاشخاص المصابين بالفطريات ، باستثناء كبار السن، وأصحاب الأمراض المزمنة، والمصابين بالسرطان، إذ كانت نسبته في مصولهم منخفضة.

الكلمات المفتاحية: اجناس الاسبيرجيلس و الكانديدا ، انترلوكين 10.

Introduction

Fungal pneumonia is considered one of the most common and deadly diseases among invasive fungi, as it affects a large number of patients who suffer from immunodeficiency and various other accompanying diseases. Yeasts and molds are the main causes of fungal pneumonia (Kontoyiamis, 2023). The genus *Candida* is the most common cause of fungal infections worldwide, as Bongomin *et.al*. 2017 pointed many species coexist harmlessly in the host's body, including humans. However, when the barriers are broken, mucous membrane or weakened immune system, can invade and cause disease, known as opportunistic infection. Candida is present on most mucosal surfaces and mainly in the gastrointestinal tract, as well as infecting the skin (Gupta *et.al* 2012). *Candida albicans* is the most commonly isolated species and can cause infection (candidiasis or thrush) in humans (Calderone *et.al* 2012). It is accompanied by some chronic lung diseases that are a trigger for infection, such as pulmonary tuberculosis, asthma, lung abscesses, as well as chronic or acute bronchitis (Al-Janahi, 2012; Al-Waisi, 2021).

Fungal infections of the lung are usually contagious and cause deaths, especially in patients who suffer from a defect in the immune system, which weakens the host's immune response to the disease (Bellocchio *et.al*., 2004; Jose *et.al*., 2012). It has been recorded that many cells and molecules are responsible for regulating the host's response to fungal infection in the lung. When the infection begins, the host's defenses are stimulated, represented by B cells, macrophages, dendritic cells (DC), and neutrophils, as a first line of defense, represented by phagocytosis and secretion. Cytokines, and natural killer cells also play a role In controlling the spread of fungi in the lung through direct and indirect killing of fungal cells, as well as the role of adaptive developing cells, including Th1 (T helper 1) and (Th17 T helper 17), they have a role through their production of IL-17 and Interferon γ (INF). γ), and immune responses vary according to the immune nature of the host, as in people with high (natural) immunity, the immune system has high resistance to invading fungi, as it works to kill and eliminate the pathogens using the cells of the lung immune system, while immunosuppressed people have high defenses. Weaker, as phagocytic cells are unable to kill and eliminate pathogens, in addition to weakness in cellular immunity represented by CD4 and T lymphocytes, which both contribute to the host's resistance to fungi (Saag *et.al*., 2000; Manavath *et.al*., 2004). Invasive Aspergillus and Candida diseases, despite the difference in the cause, the immune response to both is similar, as Th 1 and INF-γ are stimulated as an immune response to them at the beginning of the infection, as Th 1 cytokines work to stimulate and activate phagocytic cells to kill the invading fungus, as the production of INF - INF-γ is associated with Th 1 cytokines, and thus it is considered a preventive source for eliminating fungal pathogens by enhancing Th 1 production (Schroder *et.al*., 2004), so INF-γ has been used as a preventive treatment for invasive fungal infections (Lehrnbecher *et.al*., 2011; Stevens *et.al*., 2006). The second type immune response, which includes the production of Th-2 cytokines, that reduce the inflammatory reaction resulting from the production of Th-1 cytokines, as a number of different cytokines contribute to the invasive fungal infection.

TNF, IL-8, IL-6, IL-10, as IL-12 and IL-10 play an important role as regulators in the development of helper T cells and the innate immune response (Romani, 2004), but sometimes infection with invasive fungi stimulates the production of Th-2 cytokines that weaken the immune system, especially in immunosuppressed patients (Jabber., 2015). For example, when infected with the fungus *A. fumigatus*, it will stimulate the production of Th-1, but if the fungus continues to grow and form mycelium, this leads to loss of Th-1 production, stimulates Th-2 production, which leads to IL-10 production and thus leads to weak host defenses against invading fungi (Neten *et.al*., 2003).

The study aimed to identifying the fungi related to *Aspergillus* sp., *Candida* sp. that cause pneumonia, and determining the immunological role of the immune system IL10 in patients infected with systemic fungi.

Materials and Methods

Samples collection: 155 sputum and blood samples were collected from patients attending the consultation clinic for chest and respiratory diseases in Tikrit city, Salah al-Din Governorate, who suffer from a cough accompanied by sputum that persists for a long time and does not respond to treatment. Sputum samples were collected first by sterilizing the mouth and gargling with a saline solution early in the morning. A sample was taken from each patient and placed in vials. Blood samples were also drawn from each patient. The samples were collected during the period from August 2022 until December 2022.

Samples Culture : The samples were grown on Potato Dextrose Agar (PDA) medium, and the presence of fungi was initially detected by microscopic examination using 10% potassium hydroxide (KOH) by sterilized wooden swabs by passing them over the surface of Petri dishes containing the solid culture medium (PDA). The plates were incubated at 37 °C for 24 hours (Atlas, 1995). The study included the following:

Detecting the immune response to IL10: The IL10 immune response was detected through its presence in the blood serum of infected patients, using the ELISA device as in the steps below:

Standard has been added to the Wells, and the quantity of Standard must be equal to the quantity of Serum, and usually the number is 6. The enzyme was added on top of the Standard and Serum and mixed well. Then, washing solution (preservative and trace agent) was added. And finally base material was added

The purpose of using the washing solution is to get rid of unbound antibodies and other unbound foreign antigens, leaving only the required Antigen bound to the antibody present in the wall, and in this case Antigen-antibody is formed. As for the purpose of using Substrate, every enzyme needs A base material binds to it, meaning the base material binds to the enzyme, and the color of the samples changes from colorless to blue, while the wall (Well) is Its concentration is zero, meaning it does not give color. As for the purpose of Stop solution, the Stop solution (contains hydrochloric acid) is added at the end of the first minute to stop the reaction, and the color of the samples turns from blue to yellow according to the wavelength of the device, and it enters the reading stage of 450-630, the existing pits. They are antibodies, while Antigen is present in the serum in which the hormones or other components in it are to be measured.

Biostatic analysis: The Statistical Analysis System (SAS) program (2018) was used to detect the effect of the different groups (patient group and control group) on the study criteria. The T-test and LSD - least significant difference - were used to compare the means. The Chi-square was used to compare the percentages. (Probability 0.05 and 0.01) In this study, the ANOVA Tow Way program was also used.

Results and Discussion

155 sputum samples were collected from patients suffering from various respiratory infections (pulmonary tuberculosis, asthma, chest allergies, pneumonia), and the results of direct microscopic examination showed the presence of 64 positive samples, representing 41.29% of the total samples, while 91 of samples were negative (58.71%). Regarding the results of culturing the samples in SDA medium, 70 infected samples were recorded as positive (45.16%) and 85 samples were negative (54.84%) in which no growth was observed, as shown in Table (1). These results agreed with the results of Al-Ghalbi (2006), Al-Janahi (2012), Hamid (2021) and Al-Samarrai (2021), who indicated that the results of the culture examination was higher than the results of direct examination. The results disagreed with results of Muhammad(2015), as the result of direct microscopic examination was 70%, while the result of laboratory culture was 65%.

Table (1): Number and percentages of laboratory examination of samples.

Type of examination	positive samples	percentage	negative samples	Percentage	Total
Direct microscopic examination	64	41.29	91	58.71	100%
Agricultural inspection	70	45.16	85	54.85	100%
		NS Chi-Square= 2.575 P- Value 0.109			

NS* No significant differences

Through the results of the statistical analysis, no significant differences were observed, *P value* = 0.109. The reason for the appearance of negative samples may be attributed to the fact that the cause of the disease is not fungal in patients who suffer from diseases of the respiratory system. It may be due to bacterial, viral, or Genetic factors, and the reason for the difference in the results of direct microscopic examination may be due to the type of microscope used in the examination, or the reason may be due to the type of dyes used in the examination and the method used to collect the sample because direct microscopic examination cannot be relied upon in the test to detect the presence or absence of fungi (Niazi, 2000; Al-Waisi, 2021). This is what was confirmed by Collee and his group (1996). There are negative results in laboratory tests for many reasons, including diagnosis. Clinical analysis is inaccurate as it does not give accurate results, as the infection may not be fungal, or it may be due to patients infected with fungi taking anti-fungal drugs without consulting a specialist doctor.

Table (2) shows the numbers and percentages of fungi isolated during the study after conducting all the above-mentioned tests, where several species belonging to the genus *Aspergillus* were diagnosed at a rate of 68.57%, with a number of isolates amounting to 48 isolates distributed among four types, the most frequent of which was type *A. fumigatus*, where the number of isolates was 21(43.75%) followed by *Aspergillus niger*, where the number of isolates was 13 (27.08%) while the number of isolates *A. flavus* and *A .hiratsukae* were 7 for each type, at a rate of 14.58%. The results agreed with the study of Hamid (2021), which was recorded 43.18% of *Aspergillus fumigatus* and 25% of *A. niger*. The results disagreed with the study of Zohri and his group (2017), which found that the highest infection rate was recorded for *A. niger*, with 30 isolates, and15 isolates of fungus *A. fumigatus*, meaning that the number of *A. niger* isolates was higher than the number of *A. fumigatus* isolates.

Table (2): Numbers and types of molds isolated

	Mold type	N	Percentage
1	*A . fumigatus*	21	43.75
2	*A .niger*	13	27.08
7	*A . flavus*	3	14.58
4	*A . hiratsukae*	7	14.58
Total		48	100

Table (3) shows the numbers and percentages of yeasts isolated during the study after conducting all the above-mentioned tests, as the number of *Candida* isolates was 22 isolates, distributed into three species; 14 isolates (63.64%) of *C. albicans*, 4 isolates (18.18%) of each *C. parapsilosis*, and *C. dublineinsis*. The results were consistent with the study of Al-Khafaji (2021) and Al-Samarrai (2021). They found that the percentage of *C. albicans* was higher than other yeast isolates.

Table (3): Numbers and types of yeasts isolate

	Yeast type	N	Percentage
1	*C .albicans*	14	63.64
2	*C. dubliniensis*	4	18.18
3	*C .parapsilosis*	4	18.18
Total		22	100

Table (4) showed the noticeable increase in the level of the cytokine IL10 in patients infected with fungi associated with respiratory system diseases, comparing the results with healthy people (control), meaning that there are significant differences at a significant level ***p≤0.01* . It has been proven through previous studies that IL10 has a harmful effect on many pathogenic fungi (Fierer *et.al*, 1998; Vecchiarelli *et.al*, 1996).

Table (4) IL10 level in groups of patients and healthy controls

Groups	IL10 (ng/ml)
Patients	127.24±
Control	1.55±0.22
T. test	76.881**
P .Value	0.0001
	***p≤0.0*1

Interleukin 10 has multidirectional effects in regulating immunity and inflammation, as it reduces the expression of Th1 cytokines (Moore *et.al* 2001), MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. IL-10 can inhibit NFκB activity, discovered in 1991 Discovered in 1991, innate immunity, pulmonary macrophages and polymorphonuclear neutrophils play an essential role in resistance to aspergillosis. Pretreatment of mononuclear cells with IL-10 reduced the ability of the cells to damage *Aspergillus filaments*, while the phagocytic activity of conidia was increased and germination was inhibited. However, the host response to *Aspergillus* infection can be improved by reversing the immunosuppressive effects of IL-10 (Roilides *et.al,* 1997). Grunig and his group (1997) have conducted A study in an experimental model of allergic bronchopulmonary aspergillosis in which IL-10 deficiency in mice resulted in increased mortality and more severe pneumonia, suggesting a beneficial role that IL-10 plays in modulating and inhibiting the allergic inflammatory response to *Aspergillus* antigens A study in mice showed that interleukin 10 It is also produced by mast cells, neutralizing the inflammatory effect of these cells at the site of the allergic reaction (Grimbaldeston *et.al* 2007).

From Figure (1) we notice that the highest level of the cytokine IL10 is in the case of infection with *A. fumigatus* and *A. flavus*, and then infection with *A. niger*, and its lowest level is in the case of infection with *A. hiratsukae*, meaning that there are significant differences (*P<0.0001*). In the case of *Candida* infection, the highest percentage of cytokine levels was recorded with *C. albicans* infection, followed by *C. dublineinsis* infection, and the lowest percentage was recorded with *C. parapsilosis* infection with significant differences *$**P \le 0.05$* and its value is 0.0027. meaning that the analysis of ordinary variance is one-way (Bartlett's test), where the value of the corrected Bartlett statistic is 385.2 and that The *P value* varies widely

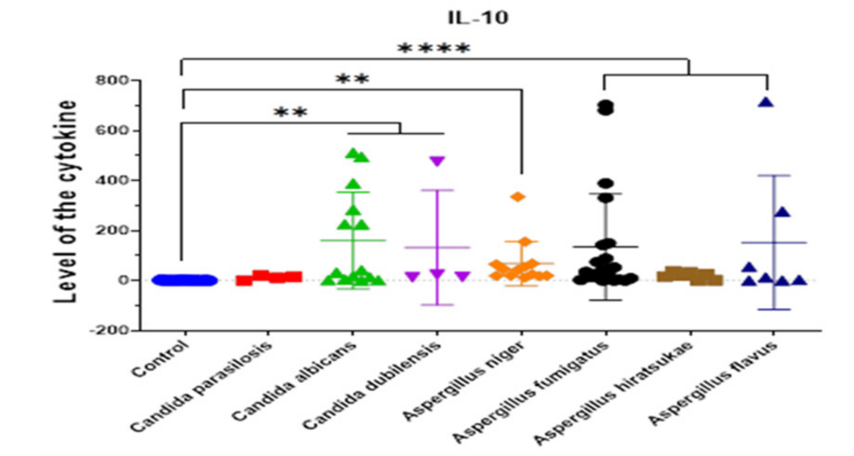

Figure (1): Values of the cytokine IL10 according to infection with different fungi

Table (5) shows that there is a difference in the concentration level of the cytokine IL10 in males compared to its concentration in females, but this difference is slight and there are no significant differences at the significant level **$p \leq 0.01$.

Table (5): Measurement of the immune variable IL10 according to gender, males and females

Groups	By gender, males and females, IL10 is a measurement of the immune variable
Males	127.75±34.96
Females	126.80±32.51
T .test	45.715NS
P value	0.984
	$P \leq 0.01$, NS No Significant **

Table (6) showed the difference in the level of the cytokine IL10 according to different age groups. There is a significant differences, **$P \leq 0.01$ and *$P \leq 0.05$, that recorded highest level with the age group between 40-50 years. There is also an increase in the number of infected people in age groups older than 50 years who secrete IL10 at a lower rate than the rest of the age groups. This is due to a weakness in their immune systems due to aging. This is also due to a weakness in the immune system due to chronic diseases such as diabetes. And others that provide opportunistic conditions that the fungus exploits to cause infection (Jassim *et.al*, 2010; Abood, 2014).

Table (6): IL10 level according to age groups in infected people

Groups	IL10 (ng/ml)
< 40 yr	116.56±31.42
40 -50 yr	183.96±62.97
> 50 yr	103.64±39.35
T .test	91.63NS
P. Value	0.404
	*$P \leq 0.05$ ** $P \leq 0.01$

Conclusion

As a conclusion of this study, *Aspergillus* and *Candida* sp. were the most common types of fungi that cause pneumonia. But the Aspergillus fungus species were higher than the Candida species , The values of the immune cytokine IL10, are higher than their normal levels in people infected with respiratory fungi. It has been found that there are differences in the values according to the different fungal species, Its level was high in the case of infection with the species *Candida albicans*, *Candida dubilensis*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus hiratsukae*, and its level was low in the case of infection with the species *Candida parapsilosis* and *Aspergillus niger*, as well as according to the ages and genders of the infected patients. We concluded that the level of the cytokine interleukin 10 was high in the blood serum of patients infected with fungi compared to healthy people. The cytokine level was similar almost in males and females, and its highest level was in the age group between 40-50 years.

References

1. Abood ,M.S.(2014). Immunological and Molecular study of Candida spp. Causing Vulvovaginal Candidiasis and the Role of Lactic acid Bacteria as Probiotic Invivo and Invitro. Ph. D .Thesis ,college of Science. Baghdad University. Iraq.
2. Al-Ghalibi, Haider Habib Hoteit (2006). Study of the effect of some plant extracts and antifungals on some skin fungal infections of pathological samples in the city of Kirkuk. Master's thesis, Tikrit University. Iraq.
3. Al-Janahi, Farqad Abdul-Ilah (2012). The effect of Cordia myxa extracts on the growth of fungi isolated from patients with pulmonary infections in the city of Diwaniyah, Master's thesis, College of Education - Al-Qadisiyah University.
4. Al-Khafaji, Dhofar Subeih Baqir. (2021). Detection of the susceptibility of Candida species isolated from cases Different clinical studies in biofilm formation. Master Thesis. The amount of education for the pure general population. Biology Tikrit University. Iraq

5. Al-Samarrai, Muhammad Amer Saad Al-Din (2021). Isolating and identifying yeasts associated with urinary tract infections in the city of Samarra and investigating the active content and biological activity of the Agaricus gennadi mushroom extract against them, Master's thesis - College of Education for Pure Sciences - Tikrit University.
6. Al-Waisi, Hadeel Abdullah Saleh (2021). Histological comparison of the lung and liver of the white mouse experimentally infected with the fungus Aspergillus fumigatus after using the antifungal amphotericin B and the alcoholic extract of the fungus Trametes sp.
7. Atlas, R. M., Williams, J. F., & Huntington, M. K. (1995). Legionella contamination of dental-unit waters. Applied and environmental microbiology, 61(4), 1208-1213.
8. Baron-Cohen, S., & Goodhart, F. (1994). The mindreading system: new directions for research. Current Psychology of Cognition, 13(5), 724–750.
9. Bellocchio S, Moretti S Perruccio K, Fallarino F, Bozza S, Montagnoli C, et al ., TLRs govern neutrophil activity in aspergillosis . J Immunol. (2004) 173:7406-15 .doi: 10.4049/ jimmunol .173.12.7406
10. Bongomin F, Gago S, Oladele R, Denning D. Global and Multi-National Prevalence of Fungal Diseases-Estimate Preci sion. J Fungi (Basel) 2017; 3: 57.
11. Calderone RA, Clancy CJ, editors. Candida and Candidiasis. Washington: American Society of Microbiology Press. 2012.
12. Collee, J.; Frase, A.; Marmion, B.& Simon, A. (1996). Makie and McGartney practical medical microbiology. 14th. Ed. Churchill Livingstone. New York. 978
13. Fierer J, Walls L, Eckmann L, Yamamoto T, Kirkland TN. Importance of interleukin-10 in genetic susceptibility of mice to Coccidioides immitis. Infect Immun. 1998;66:4397–402.
14. Grunig G, Corry DB, Leach MW, Seymour BW, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. J Exp Med. 1997;185:1089–99.
15. Gupta P, Gautam P, Rai N, Kumar N. An emerging hope to combat

Candida albicans: plantbased therapeutics. Biotechnol Int2012; 5: 85-114

16. Hamid, Kanan Adnan Abdullah. (2021). Phenotypic and molecular diagnosis of some species of the genus Aspergillus spp isolated from chronic lung infections in Saladin Governorate - Iraq, Master's thesis, College of Education for Pure Sciences - Tikrit University.
17. Jabber, Milad A. M. (2015). Cytokines profiles among immunocompromised patients with fungal infections. Ph .D .thesis College of Science, University of Tikrit.
18. Jassim, A.N. ; Majeed ,H.A and Habib, K.A. (2010). Effect of Some predisposing factors on Vulvovaginal Candidiasis. Baghdad journal for sciences , 7(3) :1087-1090.
19. Jose RJ, Brown JS, Opportunistic and fungal infections of the lung . Medicine .(2012) 40:335-9.:10.1016/j.mpmed.2012.03.013
20. Lehrnbecher T, Tramsen L, Koehl U, Schmidt S, Bochennek K, Klingebiel T. Immunotherapy against invasive fungal diseases in stem cell transplant recipients . Immunol Invest .2011;14(7-8):839-852. Google Scholar
21. Manavathu EK, Ramesh MS, Baskaran I, Ganesan LT, Chandraskar PH . A comparative study of the post-antifungal effect (PAFE) of amphotericin B, triazoles and echinocandins on Aspergillus fumigatus and Candida albicans J Infect antimicrobe Chemother . (2004) 53:386-9.10. 1093/jac/dkh066 Google Scholar
22. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001). "Interleukin-10 and the interleukin-10 receptor". Annual Review of Immunology. 19 (1): 683–765. doi:10.1146/annurev.immunol.19.1.683. PMID 11244051.
23. Muhammad, Nour Jassim (2015). Molecular characterization of some causes of skin infections in Diwaniyah Governorate and the effect of silver particles on their growth. Master's thesis, College of Science, Al-Qadisiyah University)
24. Netea, M.G., Warris, A., Van der Meer, J.W., Fenton, M.J., Verver-Janssen, T.J., Jacobs, L. E., Andersen, T., Verweij, P.E. & Kullberg, B.J. (2003) Aspergillus fumigatus evades immune recognition during germination through loss of toll-like receptor-4-mediated signal

transduction . Journal of Infectious Diseases , 188,320-326.

25. Niazi, B. H., Rozema, J., Broekman, R.A., & Salim, M. (2000). Dynamics of growth and water relations of fodder beet and sea beet in response to salinity . Journal of Agronomy and Crop Science, 184(2), 101-110.
26. Romani, L.(2004) Immunity to fungal infections Nature Reviews Immunology, 4, 1-23.
27. SAS. 2018. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
28. Saag MS, Graybill RJ, Larsen RA, Pappas PG, Perfect JR, Powderly WG, et al.. Practice guidelines for the management of cryptococcal disease . Infectious Disease Society of America. Clin Infect Dis. (2000) 30:710-8. 10.1086/313757 Google Scholar .
29. Sariguzel, F., Berk, E., Koc, A., Sav, H., & Aydemir, G. (2015). Evaluation of CHROMagar Candida, VITEK2 YST and VITEK® MS for identification of Candida strains isolated from blood cultures Infez Med, 23(4), 318–322.
30. Stevens DA, Brummer E, Clemons KV. Interferon-gamma as an antifungal .J Infect Dis. 2006; 14(Supp11):S33-S37. Google Scholar .
31. Schroder K, Hertzog PJ, Ravasi T, Hume DA (February 2004). "Interferon-gamma: an overview of signals, mechanisms and functions". Journal of Leukocyte Biology. 75 (2): 163–189. doi:10.1189/jlb.0603252. PMID 14525967. S2CID 15862242.
32. Vecchiarelli A, Retini C, Monari C, Tascini C, Bistoni F, Kozel TR. Purified capsular polysaccharide of Cryptococcus neoformans induces interleukin-10 secretion by human monocytes. Infect Immun. 1996;64:2846–9
33. Zohri AN, About-Nasr MB ,Adam m, mustafa Am ,Amer Em (2017). Impact of Enzymes and Toxin potentiqlity of four Aspergillus species to cause .Aspergillosis ,Biol ,med,(Aligarh)9:409.