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Dynamic effect of zinc on fungal infection in IBD patients

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Abstract

The current study involved 150 samples, 50 for Crohn's patients, 50 for ulcerative colitis patients, and 50 control samples from Al-Kindy teaching hospital in Baghdad. The study aim to investigate the effect of zinc on fungal infection in IBD patients We isolated and named five different yeast species, including C. albicans, C. glabrata, tropicalis C. parapsilosis, C. and C., krusi C. parapsilosis, and Aspergillus, Penicillium, Muocer, Rhizopous, Saccharomycosis, and Cryptococcus. The most common fungus isolated from IBD patients, and the proportions were as follows (andida spp.74%, Aspergillus spp.12%, Penicillium spp. 6%, Mucor spp. 3%, Rhizopus spp. 2%, Saccharomyces spp. 2% and Cryptococcus spp.1%. Likewise, the most common species was Candida albicans isolated from Crohn's patients (Candida albicans 58.54%, Candida glabrata 26.86%, Candida tropicalis 12.2% and Candida krusi 2.44%), with a highly significant difference P. value (<0.001**). The findings of Zinc showed that the mean in CD and UC patients were 45.66±2.86 mg/dL and 48.78±2.59 mg/dL compared to those found in the control group 62.43±2.93 mg/dL with highly significant differences (p≤ 0.0001**). Moreover, Zinc in early and treated IBD patients had the mean values of 48.85±4.97 mg/dL in and 42.21±2.49 mg/dL in CD and 50.61±3.27 mg/dL and 45.79±4.25 mg/dL comparing UC with the control group 62.43±2.93 mg/dL with a highly significant difference (p≤ 0.0001**). Zinc serum level has a strong impact on fungal infections in IBD patients. Also, there was a highly significant difference among all under studied. According to the results, the PCR employing the fungi starter pair (ITS1, ITS4) created various molecular sizes (510-870) bp.

Keyword

Inflammatory, Candida spp,Aspergillus .pencillium,Mucor.Rhizopus.Saccharomycsis ,Zinc,PCR.

The intestine is a muscular tube that can reach a length of five feet. The cecum is the first section, the colon is the second, and the rectum is the last section. The colon is divided into the ascending, transverse, descending, and sigmoid colons. Its length is six inches (Mahood, 2013). Both Crohn's disease and ulcerative colitis fall under inflammatory bowel diseases, a chronic, progressive and relapsing affecting the gastrointestinal tracts necessitating long-term treatment or maintenance therapies (Jebur et al., 2018; Waly et al., 2022). There are IBD key clinical forms recognized as: ulcerative colitis (UC) and Crohn's disease (CD). Yet, the UC and CD pathogenesis and etiology are not perfectly characterized, but both phenotypes are suggested to be the manifestation of complex and multifactorial processes, in which genetics, environmental impacts and immunologic abnormalities are the most significant in the promotion of excessive and poorly controlled mucosal inflammatory responses against the luminal microflora elements leading to an IBD-related tissue damages caused by a dynamic interplay between immune and non-immune cells where cytokines are crucial in the mediation (Ad'hiah etal.,2008). (Mutar,.2011; Abdul-Hussein etal.,2021). Up-regulation (IL-8, IL-12 and IP-10) and downregulation (IL-10) of cytokines are suggested to have a

role in pathogenesis of UC and CD of Iraqi IBD patients (Al-Abassi etal.,2015) (However, the etiology and pathophysiology of UC and CD. Patients with IBD are vulnerable to side effects from both their medication and the disease itself. The likelihood of developing opportunistic infections is one of these consequences (Mill and Lawrance.,2014). There is now compelling evidence connecting several fungal taxa, such Candida and Malassezia, to the cellular and molecular biology of inflammatory bowel disease (Underhil etal.,2022). Defining the fungi that are present in the human intestines (luminal and mucosal) beginning to consider how the immune system may interact with these organisms have advanced significantly in recent years (Li and Leonardi.,2019). Fungi might also play a role in IBD pathogenesis. (Sokoletal.,2017). Human can get candida when there is a flaw in the device that allows fungi to flourish. Opportunities for fungal immunotherapy emerged, including those related to organ transplantation, undergoing chemotherapy for cancer patients and managing diabetes (Ali etal.,2015). Life threating fungel diseases are now frequent substantials of the immunocomprimised host populations. Candida infection has increased when antibiotics & immunosuppressive chemotherapies appeared (Drshim eta 2005). In the last decades, there have been other Candida species dectection, like Candida glabrata, C.tropicalis, C.krusei , C.parapsilosis, C.dubliniensis, and C.kefyr (Muhsen et al., 2020). Aspergillus fungi are valuable pathogen for immunocompromised patients in both morbidity and mortality (Hamzah and Hasso, 2019). Fungi can be identified by PCR (polymerase chain reaction) regardless of whether the fungus colony has formed recently, is dead or missing certain diagnostic features(Graeser and Saunte.,2020). This technology is called duplication of a DNA piece with a specific sequence which is as individual's entire genome part outside the body of the living element in vetro by a specific polymerase enzyme when there are primers correlating with complement sequences on the DNA templates (Mullis, 1990). Trace elements are important in terms of categories. They are crucial for different physiological processes of the body and critical for the proper immune system functioning (Luk6c and Mass6nyi.,2007). Zinc (Zn) is essential in terms of micronutrient for basic cell activities like cell growth, variation, and survival. Zn deficiency reduces the adaptive and innate immune responses (Hojyo and Fukada .,2016). The nutrient uptake mechanism is a crucial factor in fungal infections. Acquiring zinc as a nutrient is critical in fungal pathobiologies (Bird et al., 2020). The pH of environment is central in zinc uptake in fungi (Wilson et al., 2021). So, the free zinc concentration in the host bodies at physiological pH (7.3−7.4) seems to be low as the majority if the zinc in intracellular and extracellular fluids firmly connect with zinc-binding proteins (Amich et al., 2014). The study aim to investigate the effect of zinc on fungal infection in IBD patients.

Material and methods

The fungal samples has been collected by using a sterile stool cap that contained (10 ml) of Carry-Blair transport media, we collected the samples. Then sample incubated in BHIB for 24 hr at 37˚C. after that, a loop full of fungal culture from incubated tubes was streaked separately into the potato dextrose agar and sabroid dextrose agar. The media of pda was incubated at a of 25 ˚C for 3 day and the media of sda was incubated at 37˚C.for overnight. Blood samples were withdrawn from all participants ELISA was conducted on determining the serum level of Zinc. For this, ELISA research kits from the USA company (BioSource).

DNA Extraction from fungal

We extracted DNA from growing candida spp colonies in its culture media by the method which Canadian Boca Scientific recommends. This method is equipped with the kit of extraction.

Statistical Analyses

This study used the Statistical Analysis System program for the detection of the effect of variance factors in research parameters. We used Chi-square test for the significant comparison between percentages in the current work and significant differences at P≤ 0.001 were found.

Results and discussion

The isolated fungi species from the studied patients with crohn's and ulcerative colon diseases were included in the study. They were isolated from the patients, which are Candida sp.,.,Penicillium sp, Aspergillus sp., Mucor

sp Rhizopus sp., sp.,saccharomyces sp .,cryptococcus sp. the most common fungus isolated from IBD patients and the proportions were as follows (Candida spp.74%, Aspergillus spp.12%, Penicillium spp. 6%, Mucor spp. 3%, Rhizopus spp. 2%, Saccharomyces spp. 2% and Cryptococcus spp.1%). Likewise, the most common species which were isolated from Crohn's patients were Candida albicans (Candida albicans 58.54%, Candida glabrata 26.86%, Candida tropicalis 12.2% and Candida krusi 2.44%), with a highly significant difference P. value (<0.001**).

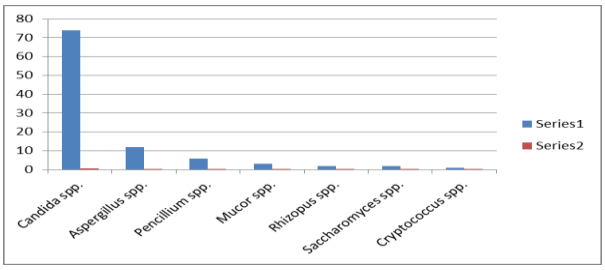

Figure1: Fungal species isolates from inflammatory bowel disease patient.

Our result about the diagnosed Candida spp is in similarity to Fritschand Abreu (2020) that detected Candida genus, the most copious element in ibd. It confirmed Trojanowska et al. (2010) in that Candida albicans were 84.4% and 81%, in respect. There are also UC and Crohn disease people, C. glabrata (8.3%), C. tropicalis (2.6%), C. krusei (2.1%). It also proved Stamatiades et al. (2018) Candida in 41 cases. IBD leads to gastrointestinal mucosal injuries, so it facilitates pathogen penetration, supporting the higher frequencies of gastrointestinal tract infections in these patients (McAuliffe, 2015). In our result, the percentage of infection with candida in Crohn's disease is higher than in ulcerative colitis. In CD patients, homeostasis between the normal intestinal flora and the host is partially broken down to evoke the components of the commensal flora as aberrant immune responses (Sands, 2007).

The recent study found that Aspergillus is the second prevalent fungi identified from ibd patients, with a 12% frequency. As seen in the Table (4-3). This finding is similar to Marti-Aguado et al. (2017). Aspergillosis was recorded in a patient with ulcerative colitis. Bradley etal.,2019 also found Aspergillus in Crohn's patients. The reason for the presence of aspergillus in those affected inflammatory bowel diseases is the weak immunity due to the intake of corticosteroids that weaken immunity and facilitate the penetration of aspergillus into the intestine (Leiferman, 2019). This supports and enhances our study. The cause of infection with Penicillium is weak immunity or a course of antibiotics taken randomly (Diekema et al., 2003). According to Zhang et al. (2016), there have been an increasing number of cases of immunocompromised patients becoming infected with Pencillium previous years. This is in line with the assertion made by Chowdhary et al. (2016)

Candida and other isolated fungi identification by ITS

The findings revealed the existence of sizes between bp 550 and 625. In accordance with the variation in lengths of the region (ITS 1 and ITS4) for yeast species, they produce various sizes of DNA fragments that enable yeast diagnosis. For instance, a type C. albicans polymerase chain reaction was for a 550 bp-sized gene. The results showed that the number of yeasts that were genetically diagnosed by its examination of two species of yeast included species belonging to the genus Candida and others belonging to other genera, as follows: C. albican, C. glabrata, while Genomic DNA isolation is conducted from fungal mycelia which previously grows on PDA. The results obtained from using the polymerase chain reaction technique showed that the two oligonucleotide Universal Primers for ITS1 (18S) and ITS4 (28S) had amplified rRNA genes to amplify the intervening 5.8S gene of the fungus by utilizing these primers Houbraken and White et al. (1990). Attempts were made to identify Aspergillus, Penicillium and Mucor at the species level. Under investigation when the results produced were analyzed. As can be seen in Figure (4-15), the amplified bands ranged between (500 - 550) bp. DNA sequences of fungi's ITS region are the most common. As a result of its great degree of variation, it has traditionally been most valuable in phylogenetic studies at the species level

Table (1) fungal resulting from Polymerase chain reaction (PCR) technique

Isolation number	Fungus	DNA Extraction	PCR Result ITS4 500-650 bp
1	Candida albicans	+	+
2	Candida gelbrata	+	+

3	Penicillium marneffei	+	+
4	Aspergillus Flavus	+	+
5	Mucor inducus	+	+

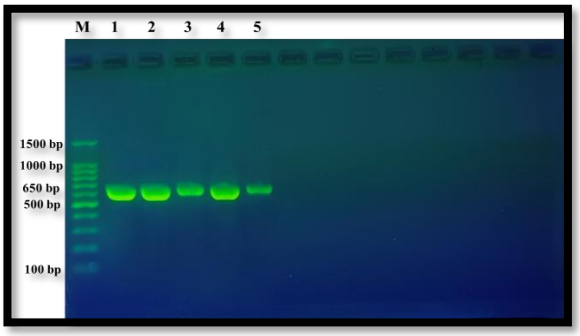

Figure (2): PCR product with the band size 650 bp which was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1hr. N: DNA ladder (100).

Zinc

The result for Table (2) showed a highly significant difference in Zinc serum level P value=0.0001among three under studied groups (crohns, ulcerative colitis and control). The results were (45.66±2.86, 48.78±2.59, 62.43±2.93) ng/L respectively, while these no significant difference between G2 and G3 P value=0.43.

Table (3): Zinc serum level among three studied groups.

Parameter	Patients Groups	Mean+S.E	P value
Zinc (mg/dL)	Control (G1)	62.43±2.93	<0.0001**
	Crohn's (G2)	45.66±2.86 [a]	
	Ulcerative colitis (G3)	48.78±2.59 [a]	
	Between two diseases		0.43 NS

As shown in Table (4), Zinc serum level recorded a highly significant difference between patients groups [crohns , ulcerative colitis (early diagnosis, treated), and control. The result were [crohns (48.85±4.97, 42.21±2.49); ulcerative colitis (50.61±3.27, 45.79±4.25)] ng/L respectively as compared to control (62.43±2.93) ng/L P value = 0.0001.

Table (4): Zinc serum level in patients groups) (early diagnosis, treated) patients and control group .

Parameter	Groups		Mean+S.E	P value
Zinc (mg/dL)	Control(G1)		62.43±2.93	<0.0001**
	Crohn's(G2)	Early diagnosis	48.85±4.97 [a]	
		Treated	42.21±2.49 [a]	
	Ulcerative colitis(G3)	Early diagnosis	50.61±3.27 [a]	
		Treated	45.79±4.25 [a]	

As shown in Table (5) which recorded a strong impact of fungal infection (candida & other fungi) on Zinc serum level in both patients (CD, UC) & control (candida infection), there was a highly significant difference among all under studied. The results were [G2 (46.79±3.45, 41.64±4.39); G3 (47.45±3.24, 51.35±4.35)] ng/L respectively, as compared to control G1 (61.53±5.37) ng/L P value = 0.0001.

Table (5): Zinc serum level in patients CD&UC (candida & other fungi) infection and G1 control (candida).

Parameter		Fungi groups	Mean+S.E	P value
Zinc(ng/L)	Control (G1)	Candida	61.53±5.37	0.0001**
	Crohn's (G2)	Candida	46.79±3.45	
		Other fungi	41.64±4.39	
	Ulcerative colitis (G3)	Candida	47.45±3.24	
		Other fungi	51.35±4.35	

Table (6) shows the impact of age on Zinc serum level in all under studied groups control, crohns ,ulcerative colitis . The age groups were (20-30, 31-40, 41-50, 51-65) years. The results for the control were (65.71±5.27, 67.27±5.08, 51.88±4.76, 54.43±6.25) ng/L respectively, crohns (46.83±4.32, 41.25±3.48,

52.57±7.14, 31.00±7.00) ng/L respectively and ulcerative colitis (44.67±4.17, 51.08±6.66, 50.33±5.97, 50.15±4.34) ng/L respectively. There is no significant difference among patients age group P value=(0.43, 0.78) while in the control age groups there was a highly significany) P value= (0.005).

Table (6): Zinc serum level distribution in all understudied groups according to age

Parameter	Age groups		Mean+S.E	P value
Zinc (mg/dL)	Control (G1)	20-30	65.71±5.27	0.005** A
		31-40	67.27±5.08	
		41-50	51.88±4.76	
		51-65	54.43±6.25	
	Crohn's(G2)	20-30	46.83±4.32	0.43 NS
		31-40	41.25±3.48	
		41-50	52.57±7.14	
		51-65	31.00±7.00	
	Ulcerative coltis (G3)	20-30	44.67±4.17	0.78 NS
		31-40	51.08±6.66	
		41-50	50.33±5.97	
		51-65	50.15±4.34	

The result of current study revealed that zinc serum level recorded significant decrease in patients compared to controls. This result agrees with the previous study done by Santucci et al. (2014), who reported the popularity of zinc deficiency in patients with Crohn's disease, in particular in those with skin lesions and growth retardations. In addition, the zinc levels usually decreases in those chronic diarrheas and malabsorptive disorders so the trace deficiency is popular in those with IBD during active disease and remission (Iwaya et al., 2011). Zinc loss increases usually with diarrhea, ostomies, and high-exit fistulas, usually in IBD and with the chronic malabsorption states with intestinal inflammation (Wong et al., 2015). As albumin transports zinc, low albumin levels, primary popular in IBD people who experience malnutrition, malabsorption rise.

In early diagnosed (CD and UC) patients, zinc decreased and the treatment group increased the level of zinc levels, but not at the required level. And the increase in zinc happens after treatment so the significance to considered zinc as a micronutrient needs monitoring in IBD people (Zupo et al., 2022).

More important result is that th fungal infection zinc recorded a significant decreasing in both patients (CD and UC) who are infected with candida and other fungi. This result is in concurred with study performed by Zhai et al. (2022) where the zinc level decreased in individuals who had an increase in Aspergillus infection because some fungal species have morel mechanisms for sequestering zinc from host cells and tissues similar to ironing chelation by secreted siderophores. C. albicans produce the antigenic proteins Pra1, a zinc-binding protein scavenging zinc from tissues which fungus occupies. Also, molecular docking experiments showed that Pra1 may interact with the zinc transporters Zrt1. Pra1 takes part in suitable endothelial colonization with C. albicans (Citiulo et al., 2012).

Conclusion

Fungal co-infection poses a significant health risk in those infected with inflammatory bowel diseases. It continues to fight IBD due to immunological issues that weaken the defenses of the body against opportunistic and true pathogenic fungi. Furthermore, the zinc deficiency was higher in IBD patients than in the controls. Its levels in early-diagnosed (CD and UC) patients were declining, and although the treatment group increased zinc levels, they did not do so to the necessary extent. Additionally, the rise in zinc following treatment underscores the need of considering zinc as a micronutrient that IBD patients should monitore. The fungal infection zinc showed a substantial decrease in both the CD and UC patients who were infected with candida and other fungi, which is a more meaningful outcome.

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