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Assessment of complement (C2&C4) in patients with recurrent candidal infections

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ABSTRACT

Background: Over the past few decades, candidiasis is a disease of growing incidence that parallels the increasing number of immunocompromised people. The entity of recurrent candidal infections has been defined as at least four symptomatic episodes at the last year. Candida albicans pathogen acts as activating surface for complement deposition i.e., covalent binding of C3b. Opsonization is the process of deposition of complement fragments on the surface of pathogen that allows their recognition, ingestion, and destruction by macrophages, phagocytic cells, neutrophils, and monocytes. IgG antibodies and C3 fragments are the classical opsonins. Phagocytes express specific receptors for C3 fragments. Complement opsonization resulting from the direct activation of the alternative pathway on pathogen surface allows their elimination by phagocytes before the mounting of an adaptive immune response and the appearance of antibodies.

Aim of the work: The aim of the present work is to clarify the relation between serum complement (C2&C4) and recurrent candidal infections.

Patients and Methods: A case control study. Sera were obtained from 50 recurrent candidal infection patients and 30 healthy volunteers. C2 levels were measured using ELISA with standard kits from EIAab R&D Systems. C4 levels were measured using Automated Chemistry ELISA with standard kits from EIAab R&D Systems.

Results: Serum complement C2 was significantly lower among the cases with recurrent candidal infection (65.63±48.35pg/ml) than in healthy volunteers 200.29± 358.43) with p-value= 0.01.

Conclusion: Recurrent candidal infection can be caused by low (C2) level in patient's serum. There is significant alteration in complement (C2) level ($P \leq 0.01$). There is no significant alteration in complement (C4) level ($P \geq 0.05$). Serum complement (C2) level can be used as a laboratory investigation for patients with recurrent candidal infections especially those with associated systemic disease.

Keywords: Recurrent candidal infections, candida albicans, complementC2, complement C4.

Conflict of interest: No financial and personal relationships between authors and others that might be perceived by others as biasing their work. Potential conflicts do not exist.

INTRODUCTION

Candidiasis is a disease of growing incidence. The number of cases increases with increasing number of immunocompromised people over the past few decades ***(1)***. The entity of recurrent candidal infections has been defined as at least four symptomatic episodes at the last year ***(2)***.

Candida albicans is the most frequently isolated yeast. In immunosuppressed patients endogenous infections emanating from colonization sites can occur with cutaneous infections, oroesophageal candidiasis and candidal vaginitis ***(3)***.

The mucosal lesions are milk curd-like loosely adherent white patches on the

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oral and vaginal mucous membranes [4]. The cutaneous lesions are clinically characteristic and often diagnostic. Distal parts of the extremities especially the hands and nails are the most common sites of skin involvement [5].

The main role of complement in pathogen elimination is indirect. Candida albicans pathogen acts as activating surface for complement de22 position i.e., covalent binding of C3b. C3b activated form of the central complement component C3. Opsonization is the deposition of complement fragments on the surface of pathogen that allows their recognition, ingestion, and destruction by phagocytic cells, neutrophils, monocytes, and macrophages. Classical opsonins are IgG antibodies and C3 fragments. Phagocytes express specific receptors for C3 fragments. Complement opsonization resulting from the direct activation of the alternative pathway on pathogen surface allows their elimination by phagocytes before the appearance of antibodies and mounting of an adaptive immune response [6].

CR3 is also the principal adhesion receptor for Candida albicans on neutrophils, macrophages and lymphocytes. The interaction between CR3 on lymphocytes and the yeast is the prerequisite for the resultant antifungal effects such as inhibition of candida hyphal growth and induction of cytokines that modulate the antifungal activity of other immune cells. The immune reactions that occur via contact of lymphocytic CR3 to Candida are probably the principal defense mechanisms on mucosal and epidermal surfaces [7].

The main consequence of phagocytosis is the elimination of pathogens. Internalized microorganisms are killed by both toxic reactive oxygen compounds and microbicidal components, such as lysozyme and proteases which present in phagocyte granules fused with the phagosome to form the phagolysosome. Phagocytic cells undergo apoptosis [8].

Another role of complement is recognition of the pathogen-associated molecular patterns by C1q in the classic pathway (CP) and mannan binding lectin (MBL) in lectin pathway (LP). C1q bind to pathogen proteins and initiate the classic pathway. Upon target recognition, C1q undergoes conformational changes and activates the two-serine proteases C1r and C1s. Activated serine proteases of the CP and LP cleave C4 and C2 to allow formation of the C3 convertase (C4b2a), which cleaves C3. If this convertase is not regulated, C3 deposition will be accelerated [9].

AIM OF THE WORK

The aim of this study is to assess the correlation between serum C2&C4 levels and recurrent candida infections.

PATIENTS AND METHODS

The present study was carried out in 80 patients They were collected from the Out-patient Clinic of Dermatology & Venereology Department, Damietta Al-Azhar University Hospital and Damietta Dermatology and Venereology Hospital, at the period from January 2018 to August 2018. **The study was approved by the Ethics Board of Al-Azhar University.**

The studied group was divided into the following groups:

Group I: 50 Patients with recurrent candida infections. Their ages ranged between 19 and 53 years 31.73± 7.98 years. They comprised 38 female (76 %) and 12 male (24%).

Group II: 30 Healthy volunteers as control group. This group comprised healthy volunteers without past, current or family history of recurrent candida infections. Their ages ranged between 16 and 47 years 30.33±8.79 years. They comprised 21 female (70%) and 9 male (30%).

Peripheral venous blood sample was obtained from patients and control subjects, collected from patients attending outpatient clinic. Serum C2 levels were

measured using ELISA with standard kits from EIAab R&D Systems. Serum C4 levels were measured using Automated Chemistry ELISA with standard kits from EIAab R&D Systems. Serum was stored at –30°C until assayed. All analyses were performed according to the manufacturer's protocol.

Statistical analysis:

Data were collected, summarized and reported on data collection sheets. Data re-entered into computer Microsoft Excel sheets with appropriate tabulation and graphical presentation. Data entry, processing and statistical analysis carried out using Med Calc ver. 15.8. Tests of significance (Chi square, student's t-test, Mann-Whitney's test, Spearman's correlation coefficient and multivariate linear regression analysis) were used. Data were presented and suitable analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. P-values less than 0.05 (5%) considered to be statistically significant.

RESULTS

Serum C2 levels in both groups

Table 1 show the median and quartile values of serum C2 level in both groups. We found significant differences between the group of patients with recurrent candida infection and control group CG (Figure 1).

Table (1): Comparison between the two studied groups regarding serum levels of C2.

C2	Study groups						T-test		
	Patients (N=50)			Control (N=30)			T	DF	P
Min – Max	4 – 144			15 – 1520					
Mean±SD	65.05	±	48.03	200.29	±	358.43	2.64	76	**0.01****
Median	84			105.43					

The statistical analysis showed that serum C2 was significantly higher in recurrent candida infection than in control group ($P \leq 0.05$).

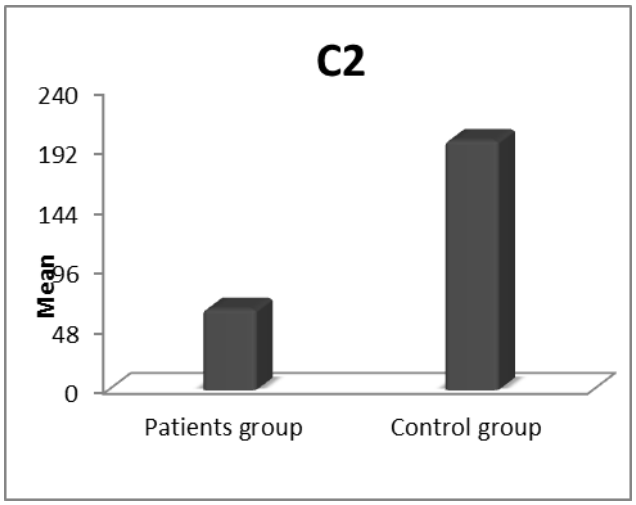

Figure (1): Comparison between the two studied groups regarding serum levels of C2

The relation between serum C2 levels and associated diseases: The statistical analysis showed that serum C2 was significantly higher in patients with asthma and patients with DM (p=0.041).

Table (2): Relation between serum C2 level in recurrent candidal infection associated with diabetes mellitus or with asthma.

C2	Study groups						T-test		
	Patients (N=50)			Control (N=30)			T	DF	P
Min – Max	4 – 144			15 – 1520					
Mean±SD	65.05	±	48.03	200.29	±	358.43	2.64	76	**0.01****
Median	84			105.43					

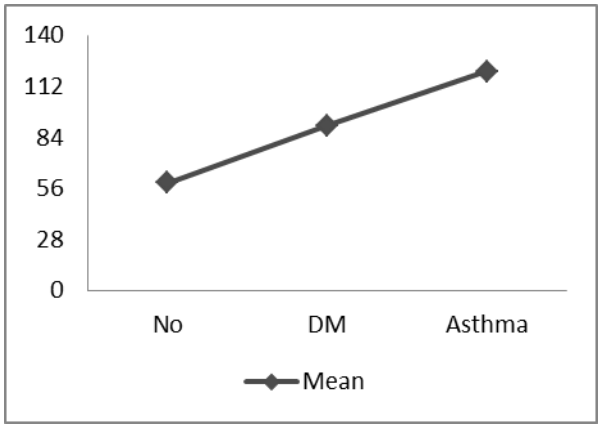

Figure (2): Relation between serum C2 level in recurrent candidal infections associated with diabetes mellitus or with asthma.

Comparison between the two studied groups regarding serum levels of C4:

Table (3): Comparison between the two studied groups regarding serum levels of C4.

C4	Study groups						T-test		
	Patients (N=50)			Control (N=30)			T	DF	P
Min – Max	67 - 304			72 - 309					
Mean±SD	137.08	±	50.00	151.73	±	59.90	1.18	76	0.243
Median	120			150.5					

The statistical analysis showed that serum C4 was insignificantly lower in recurrent candida infections than in control group (RCI vs. CG, P ≥0.05).

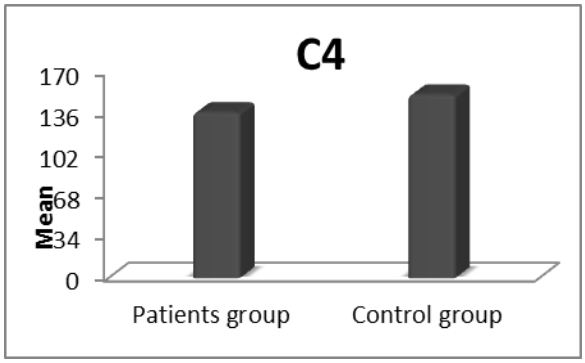

Figure (3): Comparison between the two studied groups regarding serum levels of C4.

Relationship between sex and annual frequency of disease:

Table (4) show a statistically significant relationship between sex and the annual recurrence rate of the disease with a value of 19.60 and significance level 0.012. Females were more frequent than males.

Table (4): Relationship between sex and annual frequency of disease:

Recurrence Rate / year	Sex				Chi-square Test	
	Male (N=12)		Female (N=38)			
	No.	%	No.	%	c^2	P
2	1	8.33	3	7.89	19.60	**0.012***
3	-	-	7	18.42		
4	4	33.33	4	10.53		
5	1	8.33	13	34.21		
6	3	25.00	2	5.26		
7	1	8.33	-	-		
8	2	16.67	1	2.63		
10	-	-	6	15.79		
20	-	-	2	5.26		

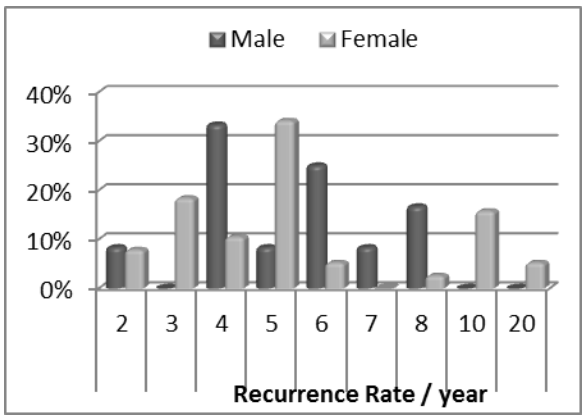

Figure (4): The annual frequency of the disease according to sex.

DISCUSSION

Candida albicans, the most frequently isolated yeast, is a ubiquitous saprophyte of mucous membranes of vulvovaginal, gastrointestinal tract, and oral cavities. In immunosuppressed patients, endogenous infections emanating from these colonization sites can occur with cutaneous infections, oro-esophageal candidiasis, Candida vaginitis and septicemia [(10)].

Candida dissemination leads to many interactions between the yeast and the immune system of the host. The powerful complement system after activation exerts a variety of antimicrobial effects to limit fungal proliferation and pathogenesis [(11)].

Fungal infections are still a problem despite all medical progress. Medical revolution such as improved antiretroviral therapy, organ transplantation, and leukemia treatment is unfortunately a factor for the growing number of immunocompromised patients susceptible for fungal pathogens[(3)].

There are several risk factors for recurrent candidal infection. Diabetes mellitus, bone marrow transplantation and hematologic malignancies are the main ones [(12)]. Foley catheter, central intravascular access devices [(13)].Corticosteroid, broad-spectrum antibiotic, immunosuppressive drugs and chemo and radiation therapy are also major risk factors for recurrent candida infections [(14)].

Host immunity might be one of the most important risk factors of recurrent yeast infections. Complement system is a very important component of the immune system.

Our current study aim to clarify the relation between serum complements' (C2) and (C4) and recurrent candidal infection. We held a comparative study enrolled 50 patients with recurrent candidal infections and 30 healthy volunteers as control group, with age ranging between 16 to 53 years old to assess the correlation between serum C2 and C4 levels and recurrent candidal infection. The mean age of all patients was 31.73±7.98 and 30.33±8.79 years in the study and control groups respectively. Regarding gender, they were (77.08% vs. 22.92 %) and (70.0% vs. 30.0 %) females and males, in the study and control groups respectively. We found that age and gender were non-significant (P > 0.05) in correlation to the recurrence of the disease.

Our study enrolled in the patients' group: homemakers (64), manual workers (24), nurses, students and teachers were the same percentage (4%) of each one. Recurrent candidal infection were more among homemakers and manual workers respectively. According to history of other diseases, we found that 8% of cases in the patient group had diabetes mellitus and 4% had asthma.

In our comparative study serum complement C2 was significantly low among the cases with recurrent candidal infection (65.63±48.35pg/ml, P ≤ 0.05), compared to control group (200.29±358.43). This result come in agreement with **Heidenreich' and Dierich**[15] that concluded that with incubation of candida albicans with sheep erythrocyte coated by C2, there was no adherence effect detectable**.**

Our study found a statistically significant difference between males and females according to average of serum level of complement (C2), where the average of C2 for the male group is 72.70 ± 53.66 and that for the female group is 62.63± 46.62 and the value of U is 202.0 and 0.555 respectively. This illustrate that females are more susceptible to infections than males. We cannot confirm this result because we did not take equal number of both genders but we got patients randomly from outpatient clinic where they were 40 female and 10 males.

In the current study, there is a difference between the serum complement C2 averages' within cases according to occupation with no statistically significant difference where the value of $\chi 2$ is 0.65 and the significance level is 0.958, indicating that there is no difference between cases by occupation categories at C2 level.

In our current study a statistically significant difference has found according to history of associated diseases, where the value of $\chi 2$ is 6.39 and the significance level is 0.041. Recurrent candidal infection is associated with diabetes mellitus and asthma, where the value was mean ± SD is 90.31± 41.37 and 120.00 ± 19.97 respectively.

In our comparative study, serum complement C4 is insignificantly low among the cases with recurrent candidal infection (135.75±50.15pg/ml, P ≥0.05), compared to control group (151.73±59.90). This result come in agreement with **Heidenreich' and Dierich**[15]. They reported that no adherence effect detected when incubat candida albicans with sheep erythrocyte coated by C4**.** Our study found a statistically significant difference between males and females according to average of serum level of complement (C4), where the average of C4 for the male group is149.08) ± 37.91 and the average of C4 for the female group is 133.29± 53.13 and the value of U is 168.0 and 0.137. This illustrate that females are more susceptible to infections than males. We cannot confirm this because we did not take equal number of both genders.

Our study found statistically significant difference between the serum complement C4 averages of cases according to occupation, where the value of $\chi 2$ is 11.31 and the significance level is 0.023. Homemakers' had the lowest level of C4. Although of this low level of C4, it

cannot has a role because we did not get a large and equal number of different occupations, also there is no medical literature concluded that there is a relation between occupation and serum complement level.

Our current study found a statistically significant relationship between sex and candidal infections distribution, with $\chi 2$ is 22.59 and significance is 0.001, where females are most susceptible to recurrent candidal infections at genital areas, intertrigo and oral thrush. Males are most susceptible to web toe and onychomycosis.

Our study found a statistically significant relationship between sex and the annual recurrence rate of the disease with a value of 19.60 and significance level is 0.012. Females are more frequent than males. . We cannot confirm this because we did not take equal number of both genders.

Our study found that there is no statistically significant relationship between the occupation and the annual recurrence rate of the disease, where the value of $\chi 2$ is 31.23 and the significance level is 0.505, but homemakers followed by manual workers are the most affected by the disease.

Our study found statistically significant relationship between the patients' history of associated diseases and the annual recurrence rate of the complaint, where the value of $\chi 2$ is 31.09 and the significance level is 0.013. The recurrence rate is more associated with diabetes mellitus then asthma. One of the risk factors of candidal infection is diabetes mellitus ***(16)t***his come in agreement with our study.

Several studies had been conducted to assess the role of different complement components in candida infection. **Liu and his colleagues(17)** concluded that cervico-vaginal concentrations of mannan binding lectin (MBL) are significantly higher in patients with vulvo-vaginal candidiasis than in non-infected individuals ***(17)*.**

Lukasser-Vogl and his colleagues (18) proved that stimulation of polymorph nuclear leukocytes (PMNs) with opsonized C albicans induce a sustained release of C6 and C7 into the cell culture supernatant within a few minutes after addition of the trigger**.**

Giraldo and his colleagues (19) illustrated additional evidence for the role of complement in antifungal host resistance via correlating between complement levels and susceptibility to Candida infections. A genetic polymorphism in the MBL gene results in reduced assembly and stability of the protein and consequently lower MBL levels in the vagina predisposes to recurrent vulvovaginal candidiasis**.** The role of complement in candidiasis has also been demonstrated in vivo by animal models. **Gelfand and his colleagues (20)** concluded that complement depletion with cobra venom factor (CVF) result in a higher mortality rate in guinea pigs with Candida infection compared to control animals**.**

In contrast to complement-competent mice, C5-deficient animals or those treated with CVF fail to develop significant neutrophilic inflammatory response after intradermal infection with C. albicans and readily permit fungal proliferation into the subcutaneous fat tissue ***(21)*.**

In patients with deficiency in the control proteins, factor I and/or factor H that associated with increased consumption of C3 when C. albicans yeast cells incubated with serum, opsonization and killing by peripheral leukocytes will reduce. This give further emphasis on the significance of the complement system on antifungal defense***(22)*.**

Ip WK and Lau Y L (23) study used freshly grown C.albicans yeasts, which incubated with or without 5mg/mL MBL and then incubated with MBL-deficient serum or heat inactivated MBL-deficient serum. The pretreatment of yeasts with MBL, followed by incubation with either MBL-deficient serum or heat-inactivated MBL-deficient serum separately, resulted

in profound inhibition of growth than when no MBL used in the pretreatment. The study documented a direct interaction between C. albicans and MBL, which result in accelerated complement activation via the lectin pathway and inhibition of growth. They concluded that, MBL has a significant influence on the host innate immunity against C. albicans without need of opsono-phagocytosis dendritic cells**.**

There are some limitations in our study, first being a comparative design, therefore we can only report on associations and cannot comment on causation, and therefore we could only make assumptions about the possible etiological relationships. Second, this study is a single center study and multicenter studies are needed to support our findings. One limitation also, the small sample size was not given a chance for the standardization of our results.

Finally in our study, we can postulate depending on our results concerning the relation between serum complement (C2&C4) and the recurrent candidal infection that the serum C2 level shown to be lower in patients with recurrent candida infection than in healthy controls. There is no significant difference on the level of C4 in recurrent candida infection patients and healthy control individuals.

CONCLUSION

In conclusion, studies of the complement activation and alteration of its components' levels by pathogenic fungi especially candidal infection provide valuable data. Recurrent candidal infection can be caused by low complement (C2) level. There is no significant alteration in complement (C4) level. Patients with systemic diseases especially diabetes mellitus and asthma are more vulnerable to recurrent candidal infection. Females are more susceptible to recurrent candidal infections at genital areas, intertrigo and oral thrush while males are more susceptible to web toe infection and onychomycosis. Serum complement (C2) level can be used as a laboratory investigation for patients with recurrent candidal infections especially those with associated systemic disease.

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