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ORIGINAL ARTICLE

Role of IL17A in Candidemic Patients in ICU at Benha University Hospital

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ABSTRACT

Background: *IL-17 plays a major protective role against fungal infection in mice and humans. Genetic defects involving IL-17 increase the susceptibility to candidiasis.* ***Objective:*** *This study aimed at assessing the association between serum Interleukin-17A (IL17A) and candida infection in Intensive Care Unit patients with candidemia versus non-candidemic patients.* ***Methodology:*** *90 individuals were included; 60 ICU patients, divided into 2 groups; group1: 30 patients with Candidemia, group 2: 30 patients with Candida in sputum and/or urine samples and 30 apparently healthy as a control group. All enrolled individuals underwent history taking, physical examination, laboratory assessment for Candida albicans infection and serum IL-17A.* ***Results:*** *IL17A level was significantly high among candidemia group (0.24 ± 0.10 pg/ml) followed by group of candida in urine and sputum (0.08 ± 0.02 pg/ml) (P-value <0.001).* ***Conclusion:*** *There was an association between IL-17A levels and candidemia suggesting its predictive value for a forthcoming invasive candidiasis.*

INTRODUCTION

Candida is a part of the microbiota of the skin, mucous membranes, gastrointestinal tract, genital and urinary apparatus of humans. There are numerous risk factors associated with candidiasis. The main risk factors are those promoting *Candida* colonization and impairment of the host's immunity**.** [1]

Candida albicans is a commensal fungus in humans, found mainly in the oropharynx, skin, and vaginal mucosa**.**[2]

The incidence of invasive candidiasis, as with other opportunistic infections, has increased in recent decades in hospitalized patients as the result of increased use of therapeutic, medical and surgical procedures. The main population groups that develop these infections are critically ill patients (mainly postsurgical patients and those with large burns)**.** [3]

The high mortality indices of the invasive fungal infections in spite of using active antifungal medications implies the presence of inadequate host immunity**.** [4]

Th17 cells play a major role in defeating fungal infections**.** [5]

Th17 cells produce several cytokines including interleukin 17A (IL-17A) such cytokines promote the activated CD4+ T cells to produce and enhance more Th17 cells IL-17A has multiple proinflammatory function. IL-17A has been demonstrated, in numerous studies which involve both mucosal and systemic candidiasis, to play a major role in the immune defense against fungal invasion**.** [6]

It is clear that early detection and appropriate management are critical in candida infection especially in immunocompromised individuals who hold a higher mortality rate. This could explain why the majority of patients (up to 70%) suffering a critical illness receive prophylactic systemic antifungal medications without any proof of the existence of such infection. So, urgent guidelines are required to guide the clinical practice towards the safest strategy for such patients**.**[7]

IL-17 stimulates many proinflammatory cytokines and antimicrobial peptides such as defensing which eliminate the extracellular bacteria and fungi, also triggers the release of the granulocyte colony-stimulating factor *(G-CSF)* as well as several chemokines which mediate the inflammatory process, including, but not limited to, *CXCL1* and *CXCL2***.** [8]

The current study aimed to assess the association between level of IL17A and candida infection in patients with candidemia in intensive care unit (ICU) versus non candidemic patients.

METHODOLOGY

The present study was conducted on 90 cases along the period between September 2015 and November 2016 after the approval of the Moral Committee of Benha College of Medicine. Each enrolled individual was guaranteed an explanation regarding the rationale,

methods, results, and complications and informed written consents was obtained from them.

Ninety individuals were included; 60 ICU patients and 30 apparently healthy persons. The patient group was divided into 2 equal groups; Group 1, 30 patients with Candidemia and Group 2, 30 patients with candida in sputum and/or urine samples. All enrolled individuals were subjected to a meticulous history taking including demographic data, duration of hospital admission, broad-spectrum antibiotic treatment and its duration, as well as, the presence of a malignancy. Also, they underwent a thorough physical examination and laboratory investigations and assessment of the serum IL-17A.

Samples:

Blood samples

Ten ml of blood were aseptically withdrawn from each patient in group1 and used as follows;

Eight ml were used for blood culture, inserted through the rubber liner of the bottle cap under complete aseptic precautions and incubated at 35-37 ° C for up to 15 days.

Two ml were used for detection of serum level of IL17A.

Urine samples

Mid-stream urine samples were collected in sterile containers or aseptically aspirating urine from port of urinary catheter.

Sputum samples:

Collection of sputum in sterile containers from endotracheal tube secretion or broncho-alveolar lavage samples.

Blood culture [9]

Strictly aseptic equipment and technique were used to collect blood samples from patients, which were then inoculated in the blood culture bottles (OXOID SIGNAL® BLOOD CULTURE SYSTEM code: BC0100) and mixed with the medium which was designed to create pressure in the sealed bottle during organisms' growth to be detected by the indicator device connected to the bottle. The growth of all organisms including aerobic, anaerobic and micro-aerophilic was detected as a rise in the level of blood/broth mixture.When the level of the mixture rises above the green locking sleeve of the growth indicator device, the result is considered positive.

Subculturing of blood cultures was done on Sabouraud Dextrose Agar (Oxoid, England). The plates were incubated aerobically for 24 to 48 hours at 37°C.

Urine and sputum samples

Samples of urine and sputum were cultured on Sabouraud Dextrose Agar, Viable candidal cell count were done (for urine samples).

Microbial growth on plates were identified conventionally

- **Identification of candida:**

Candida growth on sabouraud dextrose agar was identified by its colony morphology. Suspected colonies were examined morphologically by Gram-stain and germ tube test. [10-11]

- **ELISA** (BMS2017 and BMS2017TEN human IL-17A)

Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-17.

(Affymetrix eBioscience part of Thermo Fisher Scientific American multinational)

- ***Principle of the ELISA test:***

An anti-human IL-17A coating antibody was adsorbed onto micro wells then human IL-17A was added to the sample or standard ties to antibodies adsorbed to the micro wells. A biotin-conjugated anti-human IL-17A antibody was ties and binds to human IL-17A caught by the main counter acting agent, following brooding unbound biotin-conjugated anti-human IL-17A antibody was removed during a wash step. Streptavidin-HRP was added and bound to the biotin- conjugated anti-human IL-17A antibody.

Following brooding unbound Streptavidin- HRP was expelled during a wash step, and substrate arrangement reactive with HRP was added to the wells, a coloured product was formed in proportion to the amount of human IL-17A present in the sample or standard. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve was prepared from 7 human IL-17A standard dilutions and human IL-17A sample concentration was determined.

Statistics analysis

The gathered data were structured, tabulated and statistically examined using SPSS software (Statistical Bundle for the Sociable Sciences, version 20, SPSS Inc. Chicago, IL, USA). Box plots were performed to demonstrate median, first and third quartiles of the quantitative data. Comparability between two teams and much more was done using Chi-square test, Independent t-test was used in the comparison between two groups, One Way Analysis of Variance (ANOVA) test and Kruskall-Wallis test was used in the comparison between more than two groups

P-value was considered significant if <0.05 and highly significant if <0.001.

RESULTS

There was a significant difference between the two studied groups regarding age where the Mean ± SD of patients of candidemia group and candida in urine and sputum group was 66.13 ± 11.31 years and 54.80±16.09 years, respectively (Table 1).

Table 1: Comparison between different studied groups regarding age.

	Candidemia (n = 30)		Candida in urine and sputum (n = 30)		Control group (n = 30)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Age	66.13± 11.31	45 - 85	54.80 ±16.09	21 - 81	53.67 ± 15.51	28 -80

There was non significant difference between males and females as regard candida infection (table 2).

Table 2: Comparison between different studied groups regarding sex.

		Candidemia (n = 30)		Candida in urine andsputum (n = 30)		Control group (n = 30)		Chi square test	
		No.	%	No.	%	No.	%	X^2	P-value
Sex	**Female**	13	43.3%	11	36.7%	17	56.7%	2.509	0.285
	Male	17	56.7%	19	63.3%	13	43.3%		

Patients in candedmia group had longer duration of anti-biotic treatment and prolonged hospital stay when compared with group of candida in urine and sputum. (Figure1).

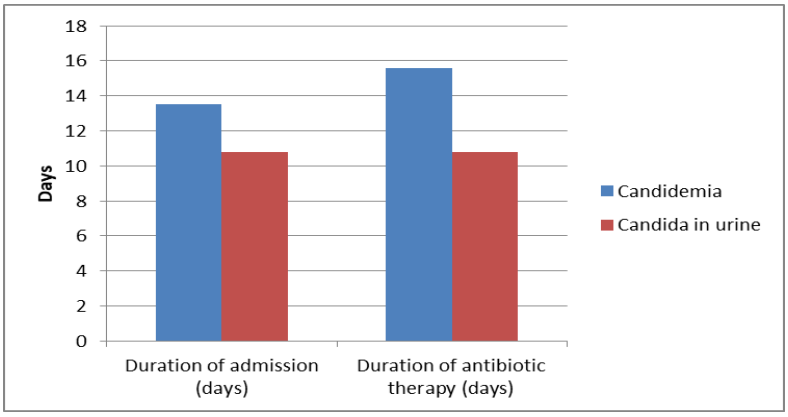

Fig. 1: Comparison between candidemia and Candida in urine and sputum regarding duration of admission and antibiotic therapy.

There was significant difference between the three studied groups regarding presence of solid tumor as, 36.7% candidimeia group had solid tumor, while no one of the two other groups had solid tumor (Figure 2).

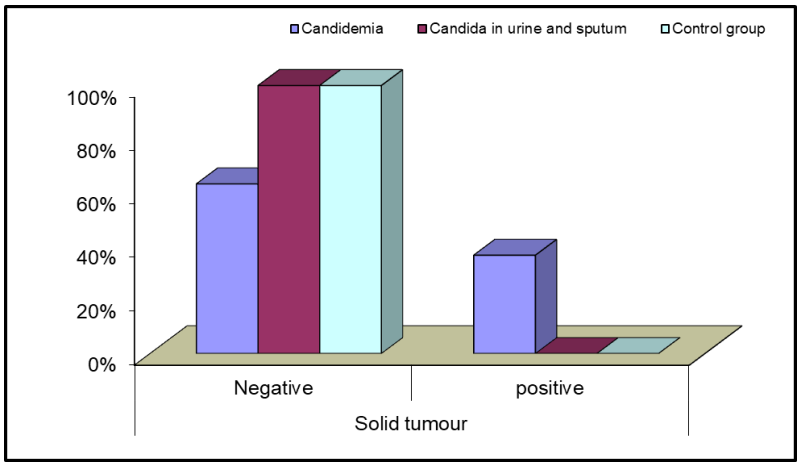

Fig. 2: Presence of solid tumor regarding candidemia group and candida in sputum and urine group.

There was an increase in serum level of IL-17A which is statistically significant between studied groups candidemia versus *candida* in urine and sputum, candidemia versus control and *candida* in urine and sputum v.s control group, as higher levels were found in the candidemia group (0.24 ±0.10 pg/ml) followed by the group of candida in urine and sputum (0.08 ± 0.02 pg/ml) (P-value <0.001). *(Table3*).

Table 3: Comparison between IL17A level in candidemia group and candida in sputum and urine group

	Candidemia (n = 30)		Candida in urine and sputum (n = 30)		Control group (n = 30)		One way ANOVA	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	f	P-value
Result of IL17A in pg/ml	0.24 ±0.10	0.104 - 0.433	0.08 ± 0.02	0.05 - 0.132	0.01 ± 0.00	0.001- 0.01	125.684	<0.001

There was a significant positive correlation between duration of antibiotic treatment and serum IL-17A (*Figure 3*).

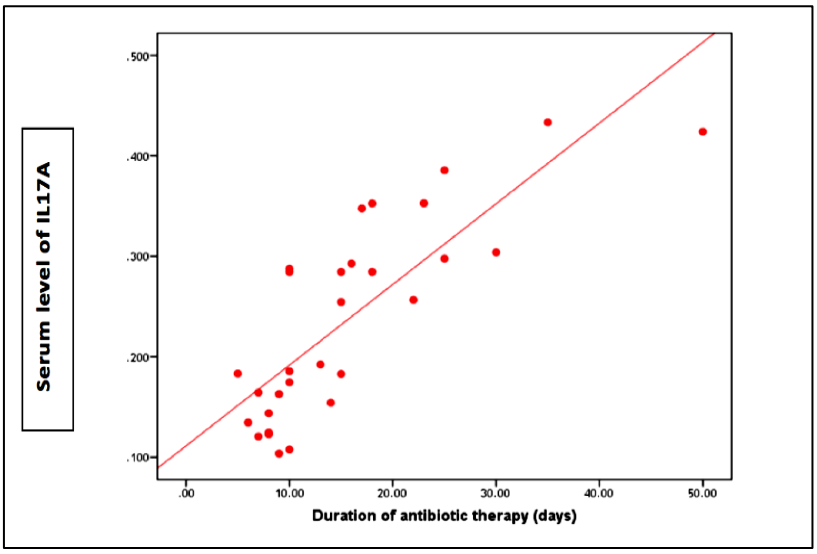

Fig. 3: Correlation between serum IL17A and duration of antibiotic therapy

Table 4: Comparison between serum level of IL17A among the studied groups.

		Serum level of IL17A in pg/ml in patients with Candidemia		Independent t-test	
		Mean in pg/ml	SD	t	P-value
Sex	**Female**	0.25	0.09	0.617	0.542
	Male	0.23	0.10		
Pneumonia	**Negative**	0.24	0.10	1.639	0.112
	Positive	0.13	0.01		
UTI	**Negative**	0.24	0.10	NA	NA
Solid tumor	**Negative**	0.23	0.10	-0.530	0.601
	Positive	0.25	0.10		
Immunocompromised	**Negative**	0.24	0.10	0.140	0.889
	Positive	0.23	0.10		

P-value of independent t- test showed non significant difference between serum level of IL17A among the studied groups (table 4).

DISCUSSION

In the current study the Mean ± SD of patients age of candidemia group and candida in urine and sputum group was 66.13 ± 11.31 years and 54.80 ± 16.09 years, respectively. The highest risk for invasive candidiasis–associated hospitalized patients progressively increased by age which is in line with Strollo and his colleagues [12] and a previously published report of population-based surveillance for candidemia. [13]

This could be attributed to many factors, for instance, the high incidence of comorbidities, aging-related physiological changes, many medication intake, and high colonization rate [14-15].

Regarding sex distribution in our study population; males represented more than half of both of the candidemia group and *Candida* in urine and sputum group. This is in accordance with the findings of Mert and his colleagues[16], as they found that 61.7% of patients diagnosed with candidemia were males.

In the present study 36.7% of the candidemia group were patients with solid tumor. Accordingly, Raza and his colleagues [17] reported that cancer patients are more liable to suffer candidemia than patients having other disorders.

In the present study we found that patients in candidemia group had longer duration of antibiotic treatment than patients with *candida* in urine and sputum group. Our results agree with the findings of Petri and his colleagues [18] as they stated that use of antibiotics is one of risk factors for infections with *candida spp*. This can be explained by the use of multiple antibiotics which suppress the bowel flora. [19]

In the current study, there was a significant positive correlation between the duration of antibiotic treatment and the serum IL-17A. Kim and his colleagues[20] assessed the IL-17 serum level before, during, and after an antibiotic therapy and reported a significantly lower IL-17 level during and after the treatment than before the treatment.

This could be attributed to the altered pathogenicity of the endogenous flora induced by the antibiotic therapy, which might require increased antibiotic selection pressure to induce a colonization of the skin and gastrointestinal tract. [21]

Also, candidemia group was associated with prolonged hospital stay when compared with the group of candida in urine and sputum. This is in accordance with the findings of Peres-Bota and his colleagues[22], as they reported that the duration of ICU stay was an independent predictor for *Candida spp*. infection.

This can be explained by prolonged hospital stay is associated with presence of central venous catheters, parenteral nutrition, antibiotic therapy, antifungal prophylaxis or surgical procedures. [23]

In the current study, there was a highly statistically significant difference between studied groups candidemia versus candida in urine and sputum, candidemia v.s control and candida in urine and sputum versus control group regarding results of IL7A, as higher levels were found in the candidemia group followed by group of candida in urine and sputum. Krause and his colleagues [24] reported a similar result in their study, IL-17A levels detected in cases with candidemia were significantly higher than those free of invasive candidiasis.

Akin and his colleagues [25] also revealed a higher level of the Th17 type cytokine. IL-17 is elevated in the serum of candidemia cases compared to both bacteremia cases and healthy individuals. The important role that Th17 conduct in the immune defense against fungal infection explains the elevated serum IL-17A levels in candidemic patients. This role was first confirmed in mice lacking IL-17 receptor (IL-17RA) which demonstrated an increased susceptibility to a disseminated *C. albicans* infection. [26]

Th17 cells secret interleukin 17A (IL-17A) in response to the stimulation of interleukin 6 (IL-6), interleukin 23 (IL-23), and interleukin 1β (IL-1β). [27]

CONCLUSION

In conclusion, candidemic patients had significantly higher level of IL-17A compared with non-candidemic patients. The statistically significant association between serum IL-17A levels and candidemia suggests its potential benefit as biomarker for anticipation of invasive candidiasis, which should be investigated in further studies.

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