Alterations in serum iron markers in allergic fungal rhinosinusitis

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Background
There is a debate concerning serum iron markers in fungal infections in patients with different diseases, and so far, none has assessed their level in fungal rhinosinusitis. Therefore, this study aimed to detect whether allergic fungal rhinosinusitis is associated with alterations in serum iron markers.

Patients and methods
Patients of this study were classified into two groups: group A included 35 patients with allergic nasal polyps, and group B included 31 patients with allergic fungal rhinosinusitis. Computerized tomography of paranasal sinuses was performed for all patients. Serum iron and total iron-binding capacity (TIBC) of all patients were measured, and unsaturated iron-binding capacity (UIBC) and transferrin saturation (TSAT) were calculated.

Results
Both the TIBC and UIBC were significantly higher in the allergic fungal rhinosinusitis group than the nasal polyps group. No significant differences were detected in the levels of serum iron and TSAT between the two groups.

Conclusion
Allergic fungal rhinosinusitis is associated with higher TIBC and UIBC, suggesting a possible role of iron in the pathogenesis of allergic fungal rhinosinusitis.

Keywords:
allergic fungal sinusitis, iron, nasal polyps, total iron-binding capacity, unsaturated iron-binding capacity

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Introduction

Rhinosinusitis is an inflammation of the mucous membrane lining the nasal and paranasal sinuses, caused by either bacterial, fungal, or viral infections or other non-infectious causes, such as allergy [1]. Allergic fungal rhinosinusitis is a distinct and frequent form of chronic rhinosinusitis seen in atopic individuals. The disease is distinguished by the presence of eosinophilic mucin containing fungal hyphae in the paranasal sinuses with the absence of invasion into sinus tissue. Despite the extensive studies conducted in the past years, many controversies exist regarding the definition and pathogenesis of allergic fungal rhinosinusitis, making it difficult to establish uniform diagnostic and management guidelines [2].

In recent years, the critical role of iron in microbial growth and pathogenesis has gained growing attention. Iron is a fundamental factor in the growth, virulence, and pathogenicity of all infectious microorganisms, including fungi. Because of its toxic effects, free iron is kept in low concentration in the body by combining with iron-binding proteins, as ferritin and heme compounds (intracellular) or transferrin and lactoferrin (extracellular) [3,4]. The low concentration of free iron in the plasma and other tissues represents a central protective mechanism against invading pathogens [5–7]. Therefore, thriving human pathogens must have mechanisms to battle with the host for its tightly bound iron. Consecutively, host defense strategies have evolved to hold iron from invasive pathogens [8,9].

The growth and survival of fungi like *Aspergillus fumigatus* and Zygomycetes in serum were reported to be associated with the removal of iron from transferrin and other iron-containing proteins, thus providing increased available free iron [5,8,10,11].

Iron uptake by fungi is achieved by specific uptake systems. In the first pathway, the ferric form is reduced to ferrous iron using cell surface reductase (ferroxidases), which is then interiorized by the high-affinity iron permease. The second pathway uses high-affinity iron-binding siderophores. Finally, a third mechanism is related to a fungal heme oxygenase, which takes up iron from heme [12].

There is a debate concerning serum iron markers in fungal infections in patients with different diseases, and

so far, none has assessed their level in allergic fungal rhinosinusitis. Therefore, this study aimed to detect whether allergic fungal rhinosinusitis is associated with alterations of serum iron markers.

Patients and methods

The study was conducted in Assiut University, Departments of Otorhinolaryngology and Clinical Pathology. The Ethics Committee of the Faculty of Medicine, Assiut University reviewed and approved the study protocol (IRB. No. 17300110). Written informed consent was obtained from all patients.

All patients with allergic fungal rhinosinusitis and patients with allergic nasal polyps admitted to the Department of Otorhinolaryngology during the period from March 2017 to September 2019 were included in the study. Patients with iron deficiency anemia, anemia of chronic disease, iron metabolic disorders, liver diseases (cirrhosis, hepatitis, or failure), or pregnancy were excluded from the study.

This study was performed on 66 patients, 46 males and 20 females. They were classified into two groups; group A included 35 patients who had allergic nasal polyps: 28 males (80%) and 7 females (20%) with a mean age of 41±14 years, and group B included 31 patients who had allergic fungal rhinosinusitis diagnosed according to the criteria of Bent and Kuhn [13]. This group included 18 males (58.1%) and 13 females (41.9%) with a mean age of 29.8±12 years.

Computerized tomography was performed for all patients using bone and soft tissue window settings, 3 mm thickness, coronal, axial, and sagittal cuts, without contrast. Lund–Mackay score was used for radiologic staging of chronic rhinosinusitis [14]. Each sinus was assigned a value of 0 (totally patent), 1 (partially opacified), or 2 (completely opacified). The osteomeatal complex was scored either 0 (not occluded) or 2 (occluded). The maximum score for each side is 12, with a total score of 24. Lund–Mackay scores of 4 or higher considerably support the clinical diagnosis of chronic rhinosinusitis, while the lower scores are indefinite [15].

Measurement of serum iron markers

Two milliliters of blood were collected on plain tubes, left for 10 min to clot, and then centrifuged at 3000 rpm for 5 min After centrifugation, the serum was separated into aliquots. Serum iron and total iron-binding capacity (TIBC) were freshly assayed in serum using Dimension® Rxl Max® integrated chemistry system. Unsaturated iron-binding capacity (UIBC) and transferrin saturation (TSAT) were calculated from [TIBC-Iron=UIBC] and [(Iron ÷ TIBC)×100=TSAT].

Results

The computerized tomographic score of the patients' groups

The radiological scores of both groups are summarized in Table 1. Scores of different sinuses were comparable between the two groups, except for the left maxillary sinus. Most of the patients scored 20 or more bilaterally.

Comparison of the levels of serum iron markers between the patients' groups

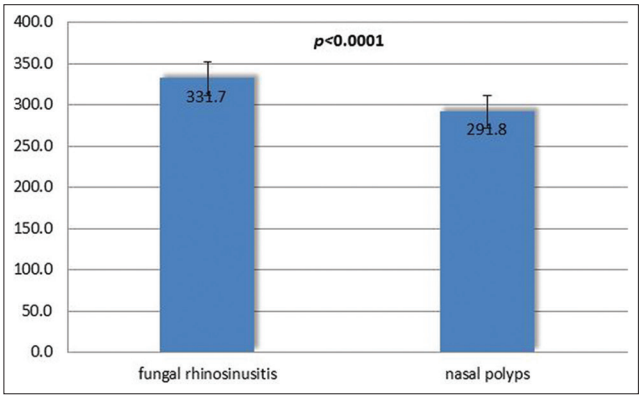
The mean serum iron level was 71.2±4.6 in patients with nasal polyps and 76.7±5.7 in patients with allergic fungal rhinosinusitis with no significant difference. Additionally, TIBC in the allergic fungal rhinosinusitis group was significantly higher than the nasal polyps group (331.7±6 vs. 291.8±7, P<0.0001). Similarly, UIBC was higher in the allergic fungal rhinosinusitis patients (255±7 vs. 220.6±8, P=0.002), with no significant difference in the TSAT between the two groups. Results are shown in Table 2 and Fig. 1.

None of the tested iron markers showed any significant correlation with the total radiologic score in either nasal polyps or allergic fungal rhinosinusitis patients (Table 3).

Discussion

There is an ongoing competition for iron among microorganisms and between the microorganisms and their hosts during infection and commensal carriage. Iron uptake during infection is needed for fungus

Figure 1



Total iron-binding capacity levels of patients with nasal polyps and allergic fungal rhinosinusitis.

Table 1 CT scores of patients with nasal polyps and patients with allergic fungal rhinosinusitis

Scores	Nasal polyps (*n*=35)	Fungal rhinosinusitis (*n*=31)	*P*
RT maxillary			
0	2 (5.7%)	3 (9.7%)	
1	7 (20%)	4 (12.9%)	
2	26 (74.3%)	24 (77.4%)	0.7
LT maxillary			
0	2 (5.7%)	4 (12.9%)	
1	10 (28.6%)	1 (3.2%)	
2	23 (65.7%)	26 (83.9%)	**0.02**
RT OMC			
0	0 (0%)	1 (3.2%)	
1	2 (5.7%)	0 (0%)	0.2
2	33 (94.3%)	30 (96.8%)	
LT OMC			
0	2 (5.7%)	1 (3.2%)	
1	2 (5.7%)	0 (0%)	0.3
2	31 (88.6%)	30 (96.8%)	
RT anterior ethmoid			
0	0 (0%)	2 (6.5%)	
1	3 (8.6%)	0 (0%)	0.09
2	32 (91.4%)	29 (93.5%)	
LT anterior ethmoid			
0	1 (2.9%)	1 (3.2%)	
1	2 (5.7%)	2 (6.5%)	0.99
2	32 (91.4%)	28 (90.3%)	
RT posterior ethmoid			
0	1 (2.9%)	3 (9.7%)	
1	1 (2.9%)	0 (0%)	0.3
2	33 (94.3%)	28 (90.3%)	
LT posterior ethmoid			
0	1 (2.9%)	3 (9.7%)	
1	2 (5.7%)	3 (9.7%)	0.4
2	32 (91.4%)	25 (80.6%)	
RT sphenoid			
0	8 (22.9%)	6 (19.4%)	
1	7 (20%)	2 (6.5%)	0.2
2	20 (57.1%)	23 (74.1%)	
LT sphenoid			
0	11 (31.4%)	5 (16.1%)	
1	7 (20%)	3 (9.7%)	0.1
2	17 (48.6%)	23 (74.2%)	
RT frontal			
0	6 (17.1%)	6 (19.4%)	
1	1 (2.9%)	3 (9.7%)	0.5
2	28 (80%)	22 (71%)	
LT frontal			
0	3 (8.6%)	6 (19.4%)	
1	2 (5.7%)	4 (12.9%)	0.2
2	30 (85.7%)	21 (67.7%)	
RT total			
0	0 (0%)	1 (3.2%)	
4	1 (2.9%)	1 (3.2%)	
6	1 (2.9%)	1 (3.2%)	
8	2 (5.7%)	0 (0%)	0.7
9	2 (5.7%)	3 (9.7%)	
10	11 (31.4%)	5 (16.1%)	
11	5 (14.3%)	6 (19.4%)	
12	13 (37.1%)	14 (45.2%)	
LT total			
0	1 (2.9%)	1 (3.2%)	
4	0 (0%)	1 (3.2%)	
5	0 (0%)	1 (3.2%)	
7	3 (8.6%)	1 (3.2%)	
8	3 (8.6%)	0 (0%)	0.6
9	4 (11.4%)	3 (9.7%)	
10	5 (14.3%)	5 (16.1%)	
11	4 (11.4%)	2 (6.5%)	
12	15 (42.9%)	17 (54.8%)	
Total			
6	1 (3.2%)	1 (2.9%)	0.5
12	1 (3.2%)	1 (2.9%)	
15	1 (3.2%)	1 (2.9%)	
16	2 (6.5%)	0 (0%)	
17	1 (3.2%)	4 (11.4%)	
18	0 (0%)	1 (2.9%)	
19	4 (12.9%)	2 (5.7%)	
20	1 (3.2%)	5 (14.3%)	
21	2 (6.5%)	3 (8.6%)	
22	5 (16.15%)	2 (5.7%)	
23	2 (6.5%)	4 (11.4%)	
24	11 (35.5%)	11 (31.4%)	

Bold value indicates significant *P* value (<0.05). CT, computed tomography; LT, left; OMC, osteomeatal complex; RT, right. Data are presented as number (percent). Chi-square test, a significant *P* value is less than 0.05.

colonization and proliferation and is thus considered a key virulence factor [16].

Serum inhibits the growth of most fungi because free iron concentrations in serum are too low to support growth. In sites where iron concentrations are low, the growth of microorganisms is allowed by the production of siderophores, which are chelating agents produced to retrieve iron from the environment [17–19]. Proteins such as transferrin and lactoferrin are utilized by hosts to gain and transport iron. Such proteins can slightly withhold the metal from the siderophores of invading bacteria and fungi [20]. In response to the invasion of microorganisms, the already free iron levels in the blood and tissue fluids of the host are further reduced through this hypoferremic response [21,22]. This competition plays a significant role in determining the fate of infectious diseases [23].

Host iron sequestration, which occurs with 'stress hypoferremia' in response to acute infection or injury [24,25], reverses this pathogenic effect and restores the antimicrobial properties of host serum [26,27]. This decrease in iron availability, or 'iron-withholding', may serve as a defense mechanism after infection or other stressful events [28].

This study aimed to detect whether allergic fungal rhinosinusitis is associated with alterations of serum

Table 2 Comparison of the serum iron markers between patients with nasal polyps and allergic fungal rhinosinusitis

Variables	Nasal polyps (*n*=35)	Fungal rhinosinusitis (*n*=31)	*P*
Iron (g/dl)	71.2±4.6	76.7±5.7	0.4
TIBC (U/dl)	291.8±7	331.7±6	**<0.0001**
UIBC	220.6±8	255±7	**0.002**
TSAT (%)	24.7	23	0.5

Bold values indicate significant *P* value (<0.05) and highly significant *P* value (<0.001). TIBC, total iron-binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron-binding capacity. Independent sample *t*-test, a significant *P* value is less than 0.05.

Table 3 Correlations of iron markers with the total CT scores in patients with nasal polyps and allergic fungal rhinosinusitis

Variable	Total score in nasal polyps	Total score in fungal rhinosinusitis
Iron	*r*=−0.2	*r*=−0.2
	P=0.1	*P*=0.2
TIBC	*r*=0.01	*r*=0.2
	P=0.5	*P*=0.2
UIBC	*r*=0.2	*r*=0.01
	P=0.2	*P*=0.5
TSAT	*r*=0.1	*r*=−0.02
	P=0.2	*P*=0.5

CT, computed tomography; TIBC, total iron-binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron-binding capacity. Kendall's Tau correlation, a significant *P* value is less than 0.05.

iron markers. Levels of serum iron markers were compared between patients with allergic fungal rhinosinusitis and with allergic nasal polyps. No significant differences were observed in the serum iron levels and the TAST between the two groups. Meanwhile, TIBC and UIBC in fungal rhinosinusitis patients were substantially higher than those in allergic nasal polyps patients.

Gifford *et al*. [29] showed that *A. fumigatus* could grow in media containing up to 80% human serum, indicating that it has an efficient mechanism for obtaining iron from serum components. Survival of *A. fumigatus* and other species in serum is associated with the removal of iron from transferrin and other iron-containing proteins [8]. This could explain the higher TIBC found in our allergic fungal rhinosinusitis patients than in the allergic polyps group. Meanwhile, finding no major differences between the two patients' groups may be explained by a previous conclusion that an elevation of TIBC occurs before the decline of the serum iron, signifying a compensatory mechanism to mobilize all tissue iron traces to maintain normal erythropoiesis.

The results of a study on 109 liver transplantation patients revealed that the levels of serum iron markers were independently associated with an increased risk of bacterial, fungal, and viral infections. That study also reported no significant differences in serum iron levels between patients who developed fungal infections and those who did not. Transferrin is the major extracellular transport protein and is usually only 30–40% saturated. Increases and decreases in tissue iron stores correspond to increases and decreases in transferrin saturation, respectively [30]. In line with their results, our findings indicate that TIBC and UIBC are more informative than measuring serum iron levels, which do not necessarily reflect the level of iron stores.

Contradictory to our results, earlier studies [31,32] reported an association between decreased TIBC and fungal infections in patients with hematological malignancies. In-vitro studies demonstrated that an increase in transferrin saturation probably promotes fungal growth [33–35] by increasing iron supply [35]. Increased transferrin saturation might be due to a decrease in transferrin concentrations without a parallel reduction in serum iron concentration as an acute-phase reactant protein response occurring in chronic disorders [32].

None of the tested iron markers showed any significant correlation with the total radiologic score in either nasal polyps or allergic fungal rhinosinusitis patients. This may be because most of the enrolled patients scored 20 or more bilaterally.

Conclusion

Allergic fungal rhinosinusitis is associated with higher TIBC and UIBC, suggesting a possible role of iron in the pathogenesis of allergic fungal rhinosinusitis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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