The possible role of chronic intestinal candidiasis in the genesis of intractable allergic diseases

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ABSTRACT
Background: A role of disturbed intestinal normal flora, such as Chronic Intestinal Candidiasis (C.I.C.) syndrome, in the genesis of intractable allergic diseases, through changing in the intestinal mucosal barrier was suggested.

Aim of the work: In this study, we have assessed the C.I.C. in the patients with intractable allergies to elucidate the potential involvement of this syndrome in the genesis of these diseases and to find out a relation between the serum level of candida IgG, IgM and IgA with the disease intractability (serum total IgE) through an intestinal functional impairment in these patients.

Materials & Methods: The serum levels of candida IgG, IgM, IgA were assessed in 20 patients with C.I.C. and intractable allergic diseases as well as in 20 healthy volunteers and their levels were correlated to the laboratory parameter of disease intractability referring to an intestinal functional impairment in these patients.

Result: When compared with the control group, the patients showed a significantly higher concentration (<0.0001) of only serum candida IgG. A statistically significant positive correlation was found between this higher concentration and disease intractability while a negative insignificant correlation was found between serum candida IgM and negative significant correlation between serum candida IgA and disease intractability in these patients. The results of intestinal biopsies showed variable lesions but there was absence of candida hyphae or intestinal invasion.

Conclusion: The results of this study suggest that disturbed intestinal normal flora produced by C.I.C. may play important role in the genesis of intractable allergic diseases. Measurement of serum candida IgG reflects the disease intractability in these patients even without increasing frequency of acute intestinal candida infection (IgM) or invasion (IgA). In addition, inhibition of C.I.C. either by drugs or diet adjustment may improve the clinical manifestations or decrease the progress of these diseases. However, further studies are needed to elucidate the exact role of C.I.C. in relation to other factors involved in the pathogenesis of intractable allergic diseases by affecting the intestinal mucosal barrier.

Keywords: chronic intestinal candidiasis (C.I.C.), allergy

INTRODUCTION
Candidiasis is incriminated in many disorders ranging from chronic fatigue to immune deficiencies1. It generally targets cutaneous or mucosal surfaces. Candida albicans is a yeast-like organism which is normally a saprophyte but can become a pathogen when shift of yeast form to mycelial (Invasive) form occurs and consequently they are considered as endogenous opportunistic pathogens2.

A number of factors are known to stimulate yeast mycelial shift. They include those which increase candidal virulence e.g. adherence and colonization depending on surface hydrophobicity; pH acidity and other colonizing organisms and enzymes productions e.g. proteinases, phospholipases, etc. as well as those which decrease host resistance e.g. immunological factors depending on natural resistance and acquired immunity and non-immunological systemic and local factors3. On the other hand, yeast
proliferation is inhibited by the normal intestinal flora, yeast nutrient-free diet, drugs like zinc, vitamin A and E, calcium pantothenate, biotin and oleic fatty acid. Candidiasis has been shown to be produced when development of clinically recognizable infection, implies failure of the mechanisms that maintain the equilibrium between the host and the parasite. This results in a wide variety of acute, sub-acute and chronic clinical syndromes due to either its proliferation at sites of normal colonization followed by its invasion or otherwise damaging tissues in these anatomic sites or due to its dissemination to tissues which are not usually colonized by candida.

Many studies suggested that Chronic Intestinal Candidiasis (C.I.C) has a role in the genesis of intractable allergic diseases and increased candida intestinal colonization have been reported in most patients than in unaffected people. In addition, it has been shown that there is cross reaction between candida albicans and food yeasts which are other major stimulators of intractable allergic diseases.

Finally, disappearance of steroids resistant allergic conditions with anticandidal treatment was described. These studies motivated us to measure serum candida IgG, IgM and IgA in patients with intractable allergic diseases and to investigate their relationship with laboratory feature of intractability in these diseases.

MATERIALS AND METHODS
This study was carried on 20 patients with intractable allergic diseases recruited from allergy and immunology outpatient clinics of Ain Shams University. They were 13 females and 7 males. Their ages ranged from 17 to 55 years. None of them had received corticosteroids or immunosuppressive drugs at the time of sampling. All the patients were suspected to have C.I.C by using a candida questionnaire and by stool testing.

The diagnosis of the patients was based on the persistence of the well-known symptoms of allergic diseases including wheezes, running nose, itching etc. as well as by the results of the skin pick tests.

Twenty completely normal individuals were also included in the study. They were 12 females and 8 males. Their ages ranged between 19 and 50 years.

All Individuals included in the study were subjected to:
1. Thorough history taking and clinical examination with stress on the allergy history for the clinical criteria of each disease and the candida questionnaire for scoring of candida overgrowth. Both history sheets were obtained from the allergy clinic of Ain Shams University.
2. Routine investigations: Complete urine analysis, CBC, ESR, etc.
4. Stool testing including stool analysis and stool culture for candida.
5. Serum total IgE detected by Elisa technique (Hycor Bio edical GinbH)
6. Serum candida IgG, IgM, and IgA detected by specific Elisa kits (Hycor Bio edical GinbH)

This assay employed the quantitative enzyme immunoassay technique in which an aliquot of sample or calibrator containing the antigen to be quantified was added and allowed to bind with solid-phase antibody. After washing, enzyme-labeled antibody was added and formed a “Sandwich complex” of solid phase antibody-Ag enzyme. Excess (unbound) antibody was then washed away, and enzyme substrate was added. The enzyme catalytically converted the substrate to product (s), the amount of which was proportional to the quantity of antigen in the sample.

For patients only: intestinal biopsies were done using small bowel Enteroscope Pentax-Video Vs P3440 by which multiple jejunal biopsies were obtained from mid-jejunal zone by enteroscopy. It was done in Gastroenterology Unit in Ain Shams
University Hospital. The specimens were preserved in formalin 10% and sectioned for microscopic examination. Selected sections were also stained with periodic-acid Schiff (PAS), *Gomori’s Methenamine-Silver* (GMS) Nitrate Stain, and Giemsa stain.

Giemsa stain is a strong stain for the characteristics of candida cells, while PAS is the most sensitive microscopic method for detecting a small number of hyphae or yeasts. This procedure results in brownish coloration of all forms of viable and non-viable fungal cells against a light background.

Sections were microscopically examined for alteration in structure of villi, mucosal ulceration pseudomembrane, hyphae and bacteria. Infiltration of lamina propria with fibroblasts, neutrophils or lymphocytes was registered in the sub mucosa and muscular mucosa observed hyphae or lymphocytes. The lymphoid follicles were observed for detecting if there is lymphoid depletion, *follicular hyalinosis*, or prominent histiocytes.

Statistical analysis was done using student t-test and ANOVA.

**RESULTS**

This study was carried on 40 subjects divided into 2 groups:

**Group I:** Included 20 patients who presented with different intractable allergic diseases and diagnosed according to the persistence of the well-known symptoms of allergic diseases and the presence of a positive skin prick test to more than one of the common allergenic extract. They were 7 males (35%) and 13 females (65%). Their ages ranged from 17 to 55 years with mean of 36±11.5 years. The duration of the disease showed a range between 1-15 years with a mean of 6.5±6.8 years.

Eighteen patients (90%) gave high grade (4+ or 3+) positive skin prick test to many of the different common allergens including candida and only two (10%) had positive skin prick test to many of the different common allergens but negative skin prick test to candida. All of them (100%) gave positive questionnaire for candida overgrowth with a score more than 60 for females and more than 40 for males.

**Group II:** Including 20 healthy volunteers as control selected from the hospital staff. They were 8 males (40%) and 12 females (60%) with ages range from 19 to 50 years mean 34±6.8 years. They were age and sex cross-matched with the patients.

Eighteen subjects 90% gave negative candida questionnaire scores and two (10%) had positive questionnaire with a score less than 60 for females and less than 40 for males.

18 patients (90%) had persistence of allergic symptoms and positive skin prick tests but none of the normal subjects had positive skin prick tests in the control group with highly significant statistical difference *P*<0.001.

There was an increase in the percentage of high score positive questionnaire in group I than in group II with highly significant statistical difference (*P*<0.001).

**Stool tests for candida**

Figure 2 shows that 90% of patients in group I showed positive stool culture for candida but only 10% in the control group with high significant statistical difference (*P*<0.001) between both groups.
Serum total IgE:

Figure 3 shows that patients had higher level of serum total IgE (Mean 181±5 µg/ml) than control group (Mean 97±37µg/mL) with highly significant statistical difference. (t-test=5.7, P value <0.001)

Figure 3: Comparison between the mean of serum total IgE in group I and group II.

In comparing patients and controls as regards candida Ig we found that:
- Candida IgG was higher in group I than in group II with highly significant statistical difference P<0.001.
- Candida IgM was higher in group I than in group II with no significant statistical difference between both groups.
- Candida IgA was lower in group I than in group II with significant statistical difference between both groups.

Regarding, the results of intestinal biopsies of patients, although none of them showed invasion, they all showed variable lesions as comprised ulcers, mucosal flecks, sloughed mucous membranes, j masses and segmental lesions. The ulcers were of variable configurations including elliptical forms (transverse and longitudinal to the bowel axis), serpiginous, linear and extensive forms with irregular margins. Segmental lesions were found in 14 cases (70%) but none of which was specific for candida i.e. no hyphae were shown. The lesions were limited to surface mucosa and sub mucosa with absence of candida invasion. (Figures 4 & 5)

Figure 4: Comprised ulcers, sloughed mucous membranes

Figure 5: Polypoid masses & segmental lesions

DISCUSSION

CIC overgrowth syndrome was first described by Branbanger and associates in 1957 and has remained a controversial subject. In normal adults, candida is a saprophyte. It can become a pathogen when shift of yeast form to mycelial or invasive forms

Table 1: Comparison between the mean of serum candida IgG, IgM and IgA in group 1 and 2.

<table>
<thead>
<tr>
<th>Candida Ig</th>
<th>Group I</th>
<th>Group II</th>
<th>t-test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida IgG</td>
<td>184±125</td>
<td>16±6</td>
<td>5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Candida IgM</td>
<td>20±17</td>
<td>16±6</td>
<td>1</td>
<td>N.S</td>
</tr>
<tr>
<td>Candida IgA</td>
<td>8±5</td>
<td>14±7</td>
<td>2.9</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*p <0.05 significant  p <0.001 highly significant

Table 2. Correlation between the mean of serum candida IgG, IgM, IgA, with serum total IgE in group 1.

<table>
<thead>
<tr>
<th>SIgE</th>
<th>181±125 µg/mL</th>
<th>Correlation Coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIgG</td>
<td></td>
<td>+0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SIgM</td>
<td></td>
<td>-0.001</td>
<td>N.S</td>
</tr>
<tr>
<td>SIgA</td>
<td>8±5</td>
<td>-0.2</td>
<td>&gt;0.05 N.S</td>
</tr>
</tbody>
</table>
form occurs by one or more factors that lead to alteration of functional integrity of the skin or mucosal barrier\(^2\). The initiation of this pathogenic shift includes candida as well as host immunological or non-immunological factors. The host factors stimulate intestinal mucosa in autocrine and paracrine manners.

CIC overgrowth syndrome has been implicated in a variety of disorders ranging from chronic fatigue to immune deficiency\(^1\).

It has also been reported that CIC overgrowth syndrome is observed in patients with impaired intestinal permeability associated with intractable allergic diseases\(^5\).

Owing to the hypothesis that deranged normal intestinal micro flora, such as CIC overgrowth syndrome are indirectly involved in the pathogenesis of intractable allergic diseases through increasing antigen invasion, we have assessed CIC overgrowth in intractable allergic diseases and correlated its presence with a laboratory parameter of disease intractability through intestinal functional impairment\(^8\)\(^9\).

The results of our study showed that serum candida IgG levels in group I were higher than in group II with highly significant statistical difference (P<0.001) while it was within normal standard range (<30 µg/ml) in the control group which means that patients had a chronic type of candida infection.

Our results showed that serum candida IgM and IgA levels were lower in both groups than the normal standard level (<30 µg/ml) with no statistical difference between them (P>0.05) for the former and this means no acute candidal infection in both groups, but with a significant statistical difference(P<0.05) for the later which means that there is no intestinal candidal invasion in both groups, but can be increased IgA secretion into the intestinal lumen but not the serum of the patients. Absence of intestinal candidal invasion was also confirmed by the absence of candidal hyphae in all intestinal biopsies of patients.

The results agree with many previous studies that reported increased serum levels of serum candida IgG in patients with allergic diseases but no significant difference in the serum candida IgA and IgM in these patients when they were compared to their control group\(^4\). Furthermore, in our study, there was a +ve correlation between this high level of serum candida IgG and serum total IgE.

Our results coincide with those of Barkhalt\(^10\) who reported a positive correlation between elevated serum levels of candida IgG and intractable disease. Regarding the insignificant correlation between serum total IgE and serum candida IgM and IgA, this was explained by suggesting that CIC overgrowth syndrome when accompanied by only increased serum levels of candida IgG, which it can be incriminated in the intractability of allergic diseases even without acute candidal infection (IgM) or candidal intestinal invasion (IgA).

Previous studies suggested that candida IgG plays a major role in allergic inflammation in patients with allergic diseases through induction of increased vascular permeability and leakage of vascular fluid into the surrounding tissue. This is clinically apparent in the mucocutaneous sites as oedema. It was also proved that antifungal treatment results in reduction of oedema as determined by Heyman et al.\(^11\).

Also, Heli and Erike speculated that increased candidal levels of IgG in patients with intractable allergic diseases were directly derived from local inflammation and hypoxia rather than from circulating candidal IgG and that the multiplicity of affected organs in these patients including the highly vascular intestinal wall allowed the immediate access of candidal IgG into the circulation through increasing permeability\(^12\).

It is speculated that the prevalence of candida infection may be the result of the widespread use of antibiotics, particularly
CIC overgrowth syndrome is a disease characterized by the production of a large number of toxins which get absorbed into the blood stream and which may be responsible for the destruction of intestinal mucosal integrity causing liver toxemia. The later allows multiple allergens to enter the circulation. Also some of these toxins destroy enzymes needed for cell energy causing the release of free radicals and lowering the intracellular pH up to 7 which is a favourite environment for the growth and proliferation of the yeast. Consequently, candida proliferates in the intestines, it can change its anatomy and physiology from the yeast-like form to the mycelial fungal form. The yeast-like state is a non-invasive, sugar fermenting organism whereas the mycelial fungal state produces very long root-like structures which can invade the mucosa. This penetration breaks down the boundary between the intestinal track and the rest of the circulation and introduces many antigens into the blood stream, such as incompletely digested dietary proteins. This explains why many individuals who have chronic candida overgrowth show a wide variety of food and environmental allergies. These antigens can powerfully assault the immune system producing a wide variety of allergies with symptoms.

Penetration of candida roots to GIT mucosa also lead to penetration of candida spores into the blood. However because the later is always alkaline, the spores can’t grow past their first to fourth stage of development.

Consequently, treatment of C.I.C. overgrowth syndrome depends upon either or both inhibition of yeast proliferation and/or enhancing healing of intestinal mucosa. The former is reached by rebuilding the intestinal flora or by giving a special diet regimen composed of low sugar, high fiber diet, yeast free and yeast nutrient poor diet.

Rebuilding of intestinal flora was done by inoculating the bowel with proper symbiotic producing bacteria such as acidophilus and bifidus based on the fact that normal flora of GIT leads to reduction of the compatibility of the intestinal environment for yeast proliferation. However, very recently, several meta-analysis studies were conducted and concluded that although the authors previously reported a reduced atopic, IgE sensitization and cumulative incidence of infant eczema, by early feeding of specific strains of probiotics, prebiotics or symbiotics, during weaning, there was no reduction of respiratory allergy. Also, this was shown by long term effects at school age, of these agents on the prevalence of any diagnosed allergic disease, airways inflammation or established IgE sensitization.

The high fiber diet increases the absorptive surface area of the faecal material and hastens the elimination of metabolic byproducts. It is used from 1-6 mgs according to the severity of the infection.

The yeast nutrient free diet which contains for e.g. grape fruits, seed extract, garlic acid etc. and the yeast free diet are prescribed by Dr. Diaa Soliman.

Enhancing healing of intestinal mucosa is done by bland’s program using high levels of Zinc (30-50mg/day), vitamin A (25,000 to 30,000 units/day), vitamin E (400-800 i.u./day) and calcium pantothenote (200 to 1000 mg/day). Biotin and the fatty acid oleic acid can prevent the conversion of the yeast form of candida to its mycelial fungal form. Bland’s program suggests biotin orally 300mcg taken three times daily along with two teaspoons of olive oil taken 3 times daily.

Finally, to conclude, we summarize that, although the aetiopathogenesis in some intractable allergic disease in term of its link with C.I.C. overgrowth syndrome is still unsettled, there appears to be a relation. Our results, like those of other studies, suggest that measurement of candida IgG, IgM and IgA in patient with C.I.C. overgrowth syndrome can be useful in the evaluation of disease intractability.
with or without acute candida infection IgM and/or invasion in these patients (IgA) and we recommend if these two later are abnormal, stool culture for candida and intestinal biopsy should be done to confirm acute intestinal infection and invasion. Also, the detection of candida IgA in the stool of these patients to confirm that the low levels of IgA which was found in these patients, was due to its secretion into the intestinal lumen to eliminate candida overgrowth. Again, as a balanced gut microbiota is crucial for the development of healthy immune regulation and gut barrier function to allow brisk immune response to pathogens and systemic hyporesponsiveness to harmless antigens such as food, the potential of specific probiotic strains to alleviate food allergy resides in their ability to modify antigens, repair gut barrier functions, balance altered microbiota and restore local and systemic immune regulation, and in patients with multiple food allergies, induction of oral tolerance by specific probiotics continues to attract research interest.

However, it should be mentioned that a need for integrated research into the basic mechanism of regulatory control of the intestinal barrier function and understanding what crosses this boundary is critical to the new understanding of food allergy and the disease it causes.

REFERENCES


